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Approaches to Golden Algae Control: In-Lake Mesocosm Experiments

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Abstract

Prymnesium parvum, a haptophyte alga, occurs worldwide. It is tolerant of large variations in temperature and salinity, and is capable of forming large fish-killing blooms. In the United States, the first recorded *P. parvum* bloom occurred in 1985 in a semi-arid region of the country (Pecos River, Texas). Since then, the reported incidence of *P. parvum* blooms dramatically increased in the United States, where the organism has invaded lakes and rivers throughout southern regions and most recently has moved into northern regions. Fortunately, *P. parvum* population dynamics are influenced by several factors that may serve as tools for management. These include hydraulic flushing, pH, and ammonia additions. This report documents in-lake enclosure experiments conducted during periods of bloom initiation, and bloom development and decline. Three approaches are demonstrated to have promise in controlling blooms in localized areas of lakes. Most promising were manipulations involving pulsed hydraulic flushing (30% water exchange once per week using water deeper in the lake); the neutralization of ambient waters (lowering pH to 7); and ammonia additions (elevating to 40 µM).

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Preface

This technical report was developed to convey the experiments and results of the Testing Approaches to Golden Algae Control: In-lake Mesocosm Experiments project on Lake Granbury, Texas. This project was supported by Congress with funding through the U.S. Army Corps of Engineers. This report was published by the U.S. Army Engineer Research and Development Center (ERDC).

The report was prepared by the project scientists, Drs. Daniel Roelke of Texas A&M University, Bryan Brooks of Baylor University, and James Grover of the University of Texas at Arlington. The Lake Granbury project was managed by the Texas Water Resources Institute (TWRI), part of Texas AgriLife Research, the Texas AgriLife Extension Service, the College of Agriculture and Life Sciences at Texas A&M University, and project manager Danielle Kalisek provided assistance in preparing the report. Dr. B.L. Harris, acting director of TWRI, was the principal investigator of the project.

Additional project collaborators included the Texas Parks and Wildlife Department, Texas Commission on Environmental Quality, and Brazos River Authority.

At the time of publication of this report, ERDC Commander was COL Kevin J. Wilson. ERDC Director was Dr. Jeffery P. Holland.

1 Introduction

Background

Inflows and salinity have long been recognized as factors influencing phytoplankton community dynamics and structure (Ketchum 1951, 1954). The magnitude and timing of inflows produce nutrient pulse and flushing loss variations that select for species adapted for these conditions, which in turn influence plankton community composition and productivity (Roelke et al. 2003; Buyukates and Roelke 2005; Miller et al. 2008). Nutrient pulses and flushing losses associated with inflows also have been linked to the incidence of harmful algal blooms (Seliger et al. 1970; Paerl 1988; Jacoby et al. 2000; Moustaka-Gouni et al. 2006; Mitrovic et al. 2008), including toxic blooms of *Prymnesium parvum* (Roelke et al. 2010a, 2011).

P. parvum, a haptophyte alga, occurs worldwide. It is tolerant of large variations in temperature and salinity, and is capable of forming large fish-killing blooms (Lundholm and Moestrup 2006; Baker et al. 2007, 2009; Southard et al. 2010). In the United States, the first recorded *P. parvum* bloom occurred in 1985 in a semi-arid region of the country (Pecos River, Texas) (James and De La Cruz 1989). Since then, the incidence of *P. parvum* blooms dramatically increased in the United States, where the organism has invaded lakes and rivers throughout southern regions and most recently into northern regions (Figure 1, from Roelke et al. 2011)). *P. parvum* blooms typically occur in aquatic systems that are eutrophic and brackish (Kaartvedt et al. 1991; Guo et al. 1996; Roelke et al. 2007, 2010a, 2010b).

Many factors contribute to *P. parvum* bloom formation. They include production of chemicals toxic to grazers (Granéli and Johansson 2003; Tillmann 2003; Barreiro et al. 2005; Michaloudi et al. 2009; Brooks et al. 2010), use of alternative energy and nutrient sources through mixotrophy and saprophytic nourishment (Nygaard and Tobiesen 1993; Skovgaard and Hansen 2003; Lindehoff et al. 2009), suppression of competitors through allelopathy (Fistarol et al. 2003, 2005; Granéli and Johansson 2003; Roelke et al. 2007; Errera et al. 2008), and resistance to the allelopathic effects of other algae (Suikkanen et al. 2004; Tillmann et al. 2007). Factors that negatively influence *P. parvum* population density might include grazing by

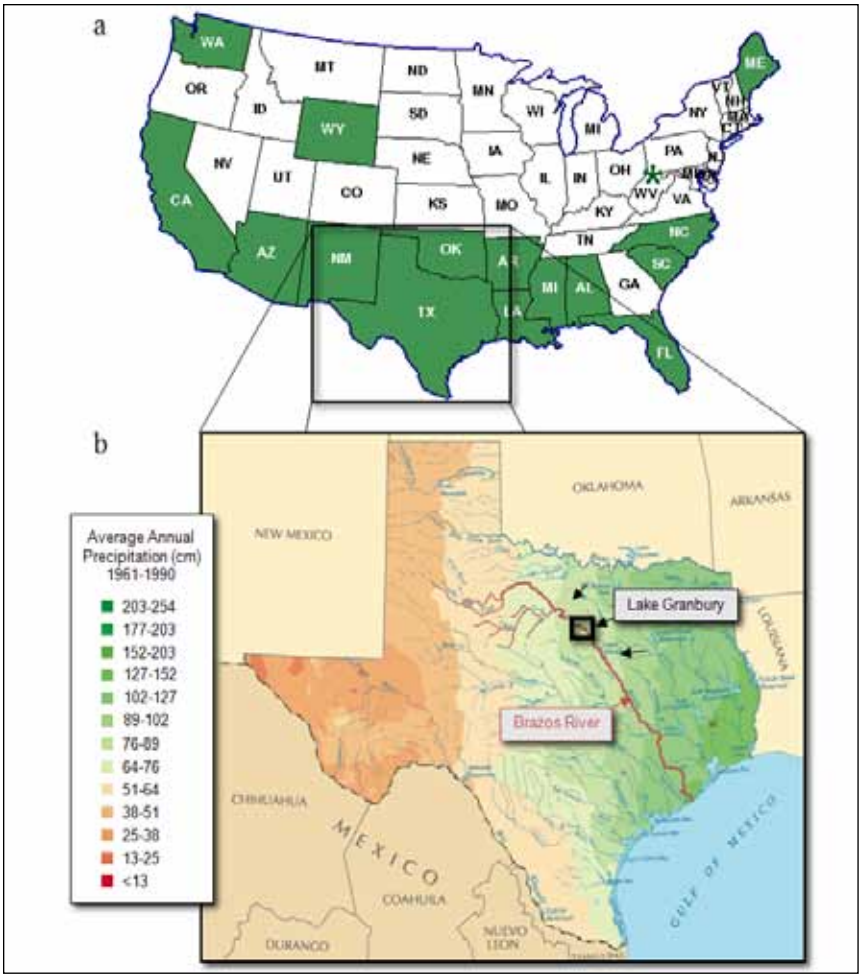

Figure 1. Distribution of *P. parvum* in the United States, where states colored green have reported the organism (a), and the location of in-lake experiments (b), which were conducted February and March, 2010.

toxin-resistant zooplankton and pathogenic effects of virus (Schwierzke et al. 2010). In addition, some cyanobacteria may inhibit *P. parvum* blooms (Grover et al. 2010; Roelke et al. 2010b; James et al. 2011).

Inflow and salinity have both been shown to be important factors influencing *P. parvum* population dynamics and reproductive growth rates. A recent study documenting the entire seasonal *P. parvum* bloom cycle in a Texas lake found that cell loss through hydraulic flushing during a period of high inflow, along with cessation of toxin production associated with nutrient loading, was the primary mechanism terminating the bloom (Roelke et al. 2010a). Another study employing data records spanning a decade for three lake systems of the Brazos River showed that inflow bloom thresholds exist. Fish-killing, system-wide blooms were not observed above these thresholds (Roelke et al. 2011). Using a Texas strain of *P. parvum*

(UTEX LL 2797), the optimal salinity for reproductive growth was determined to be 22 psu. At 10°C, a temperature representative of winter conditions when blooms are most common in the region, growth rates decreased ~10-fold (from ~0.2 to 0.02 d^{-1}) as salinity decreased from the optimum to levels found in Texas lakes (Baker et al. 2007, 2009). Baker et al. (2007, 2009) also suggested that small variations in salinity at low levels determine whether reproductive growth is possible.

Recent research group findings also identified a pH-dependent influence on the magnitude of ambient *P. parvum* toxicity in Lakes Granbury and Whitney, and *P. parvum* laboratory cultures (Valenti et al. 2010). Specifically, Valenti et al. determined that higher pH levels of 8.5 result in greater toxicity than lower pH levels (6.5, 7.5), which suggests the toxins released by *P. parvum* may behave as weak bases in aqueous solutions. These results suggest that targeted reductions of pH can reduce the potency of *P. parvum* toxins and related impacts to fisheries.

Barkoh et al. (2003) experimentally manipulated ammonia to treat *P. parvum*. These observations, when accounting for the more toxic unionized form, indicated potential utility of employing ammonia amendments for managing *P. parvum* threats to aquatic life. More recent studies by the research team (Grover et al. 2007) further examined ammonia treatments in laboratory studies. These previous efforts provide a reasonable justification to perform field-oriented experiments in confined regions of reservoirs (e.g., coves) experiencing *P. parvum* blooms to further test the effectiveness of ammonia pulses in bloom mitigation, while also examining relative effects on other components of phytoplankton and zooplankton communities.

Objective

In-lake enclosure experiments were performed to investigate potential management options for mitigation of *P. parvum* impacts on inland waters. Specifically, hydraulic flushing, pH, and ammonia were manipulated, as described below.

Approach

Study region

The Brazos River flows southeast across Texas (USA), spanning a rain gradient from the arid western regions of the state (averaging

~13-26 cm/year^{-1}) to the moister eastern region, with an average of ~155 cm/year^{-1}. This study was conducted in Lake Granbury (centered at 32.40° N, 97.76° W, construction completed in 1969), located in a region of the watershed receiving ~90 cm year^{-1} of rainfall (Figure 1). Lake Granbury is sinuous, with shorelines that follow the submerged river channel. Its capacity is 189 x 10^6 m^3. Its surface area, average depth, and total drainage area are 34 km^2, ~5.5 m, and 41,732 km^2. Lake Granbury has experienced recurrent fish-killing, system-wide *P. parvum* blooms over the last decade (Roelke et al. 2011). Locations of the fixed sampling stations are shown in Figure 2.

Figure 2. Locations of fixed sampling stations in Lake Granbury, TX (as reported in Roelke et al. 2010a), where paired stations are the paired shallow water and deep-water stations. In the following figures, data from the deep–water stations are shown.

Approach

Collaborative research focused on testing the efficacy of various approaches to controlling *P. parvum* blooms. The investigation assessed *P. parvum* bloom dynamics, and plankton community response as a whole, by measuring the response of the natural phytoplankton community to

experimental manipulations and by assessing the toxicity response of *P. parvum* under these changed conditions.

The results provide an understanding of how *P. parvum* populations respond to direct intervention (see experimental treatments below). Relative toxicity tests established linkages between toxicity and these approaches to bloom control. The research provides a better understanding of what approaches should be pursued as management tools in specific regions of Texas lakes, possibly serving as bloom control mechanisms.

Methods

Experiments were conducted at the mesocosm scale using pre-designed limnocorrals supplied by Aquatic Research Instruments. A limnocorral array was constructed that was comprised of 1.57-m^3 clear plastic, roughly cylindrical containers that extended from the surface to a depth of 2 m, with an approximate diameter of 1 m. Each limnocorral had rigid supports at the surface and bottom, a closed bottom, and a separate flotation collar. Bags were open to the air during the deployments. This design was successfully employed previously in a Texas lake similar to Lake Granbury (Roelke et al. 2007). Previous work with limnocorrals of this dimension demonstrated that the contained volume was well-mixed. This has been verified by collecting profile data within previously deployed limnocorrals. A well-mixed state was maintained because wave energy experiences little dissipation as it passes through the flexible material of the limnocorral siding. Because limnocorrals of this dimension remain well-mixed, grab samples were taken from a single depth (0.5 m). Treatments (see below) were randomly assigned to limnocorrals and implemented every seventh day of the 21-day experiments, i.e., at T0, T7, and T14. Sampling of the limnocorrals occurred on each of these days, as well as at t21 (experiment conclusion). For t-zero, sampling occurred after treatment initiation. This enabled characterization of the initial condition in each of the limnocorrals. For T7 and T14, sampling occurred just before each treatment. This enabled characterization of the effect of the treatments during the first seven and 14 days of the experiments. The first experiment was initiated on February 23, 2010, and the second experiment was started on March 31, 2010.

In-field experiments were designed to determine how *P. parvum* populations and the overall plankton community responded to manipulations that included:

Treatments (all performed in triplicate)

1. Hydraulic flushing
2. Reduced pH
3. Ammonia pulses

Hydraulic flushing was tested at three levels (0.05, 0.1, and 0.3 d^{-1}), where the control enclosure received no inflows. Controlled flushing events occurred every seventh day of the 21-day experiment (i.e., T0, T7, and T14), where days between flushing events experienced no inflow. Thus, this treatment represents a pulsed flushing condition. Flushing events were achieved by removal of a measured volume from targeted limnocorrals using calibrated buckets, followed by refilling using waters pumped from depth (~7 m). Temperature, pH, and salinity were very similar between surface and deeper waters, as this system is a shallow, warm-monomictic lake that is holomictic during winter months. Multiple water pumps, powered by an array of deep-cell 12-V batteries housed on the research vessel, were employed.

The influence of reduced pH was evaluated at three levels, 7.0 (neutral), 7.5 (slightly basic), and 8.0 (slightly reduced from natural basic condition), where the pH of the control enclosure was what naturally occurred in the lake, which was ~ 8.5 (Roelke et al. 2010a). pH of limnocorrals in Lake Granbury were manipulated to test the hypothesis that lowering pH decreases the magnitude of ambient toxicity to fish and other aquatic organisms, which extends the previous observations in the laboratory with cultures and pH manipulation of field samples to an experimental field study (Valenti et al. 2010). To examine this question, common U.S. Environmental Protection Agency (US EPA) methods for Toxicity Identification Evaluations were employed (US EPA 1991).

The pH of limnocorrals was adjusted by careful additions of 1 N sulfuric acid solution. Sulfuric acid was selected to reduce pH rather than hydrochloric, nitric, phosphoric, or carbonic acid because SO_4^- is relatively biologically benign compared to the other conjugate bases. A 1 N solution was prepared by adding 28 ml of ACS-grade concentrated sulfuric acid per 1 L of Lake Granbury water (American Public Health Association (APHA) et al. 1998). Chlorine can be highly toxic to aquatic life, which is particularly relevant for these experiments because free chlorine (the most toxic form) becomes more predominant in aquatic solutions at lower pH (Brungs 1973). Nitric

and phosphoric acid were avoided because augmenting limnocorrals with these additions could alter the nutrient (N:P) stoichiometry of the system. Similarly, carbonic acid was not selected because it would introduce more inorganic carbon into pH-adjusted systems.

For T0, the amount of solution required to reduce the pH to the desired treatment level was based on an extrapolation from a prior laboratory titration experiment with Lake Granbury water. Before any pH adjustment, the pH of each limnocorral was measured using YSI 600 XLM or YSI 6600 data sondes. Two point calibrations (pH 7 and pH 10) were completed for the data sondes prior to their use and immediately following their use as a post-calibration quality control measure. To adjust pH, a volume of 1 N sulfuric acid solution was measured in a 500-ml graduated cylinder and then slowly added in small pulses of 10-20 ml. Concurrent with the addition, surface water in the limnocorrals was mixed continuously and the pH was continuously monitored and measured with a data sonde. To ensure uniform mixing while adding the acid solution, pH was measured at various depths in the water column. Once the pH stabilized, additional sulfuric acid solution was added as previously described until the desired pH treatment level was reached. In addition to biweekly visits to each of the targeted limnocorrals for pH measurement and adjustment, data sondes were deployed in each of the pH treatment levels (low, medium, high, and control) to allow continuous monitoring of pH during the course of the experiment. Preliminary studies with pH manipulation of Lake Granbury water indicated that pH treatment levels remained relatively stable under field conditions.

Ammonia pulses were achieved through the weekly addition of NH_4Cl at prescribed levels, to triplicate limnocorrals. The two levels delivered achieved added concentrations similar to those used in previous laboratory experiments (Grover et al. 2007), levels of 10 and 40 μM. The high dose proved effective in lowering the toxicity of laboratory cultures of *P. parvum* grown at 20° C. Chloride rather than sulfate was used as the carrier anion for NH_4 additions because Lake Granbury is in a brackish stretch of the Brazos River where chloride is the dominant anion. In historical data, chloride concentrations are approximately twice those of sulfate, and constitute about 40% of total dissolved salts (Wurbs and Lee 2009). From the salinities measured during the experiments, background chloride concentrations were estimated to be at least 4900 μM. Therefore, the cumulative amount of chloride added in high NH_4 treatments would be less

than about 3.5% of the estimated background concentration. During the time period of the proposed experiments, temperature was expected to rise from about 10° C to about 20° C, so effective reduction of toxicity in the second phase of experiments is expected. Moreover, the mechanism of toxicity reduction reported earlier (Grover et al. 2007) appeared to be related to a feedback cycle whereby growing populations of *P. parvum* raised the pH sufficiently to deprotonate NH_4 to free NH_3, to which *P. parvum* is highly susceptible. A similar feedback is anticipated in these field experiments, based on previous observations of high pH (> 8.5 at some stations) during a *P. parvum* bloom in the first quarter of 2007 on Lake Granbury.

The enclosures were sampled at the start of the experiment, then every seventh day thereafter. A second 21-day experiment was initiated shortly after the first experiment was finished, employing the same treatments. The first experiment was conducted during a period of bloom initiation and the second experiment during a period of bloom development and decline. Response variables sampled for during each time of sampling included: *P. parvum* population density, phytoplankton biomass and composition, zooplankton biomass and composition, inorganic nutrients, toxicity, and other water quality parameters. These are discussed in more detail in the following section. Samples for *P. parvum* population density, phytoplankton biomass and composition, and inorganic nutrients were taken from a 1-L grab sample. Samples for zooplankton biomass and composition were taken from a 12-L grab sample, and grab samples for toxicity were 5 L. Other water quality parameters were measured on site.

To better interpret experimental results within the context of the natural in-lake conditions, monitoring of Lake Granbury continued during the period of the experiment and shortly thereafter. As part of the monitoring, 10 fixed location stations in Lake Granbury were sampled, as described previously (see Roelke et al. (2010a)). All parameters measured in the enclosures were also measured at each of the in-lake stations. To complement fixed-station characterizations of *P. parvum* population dynamics, and phytoplankton as a whole, spatial patterns of chlorophyll *a* were measured during each sampling trip with Dataflow, a high-speed, flow-through measurement apparatus developed for mapping physicochemical parameters in shallow aquatic systems (Madden and Day 1992). This integrated instrument system was used to concurrently measure multiple water quality parameters that included chlorophyll *a* (*in-vivo* fluorescence) from a boat following closely

spaced transects (see Roelke et al. (2010a)). Measurements were taken at 2-second intervals from ~20 cm below the surface. An integrated GPS was used to simultaneously plot sample locations. GPS and Dataflow information were then used to create detailed contour maps (Surfer v8.0). Monitoring of in-lake conditions occurred monthly.

P. parvum population density in surface waters was estimated using a settling technique (Utermöhl 1958), where a 100-mL phytoplankton sample was collected at ~0.5-m depth and preserved using glutaraldehyde, 5% v/v. A 1-mL subsample was then settled for 24 hr. Randomly selected fields of view were then counted until >200 *P. parvum* cells were enumerated (20 to 40 fields of view).

Total phytoplankton biomass (approximated using chlorophyll *a*) and biomass of taxonomically aggregated phytoplankton groups (approximated as a fraction of the total chlorophyll *a*) were determined from phytopigment biomarker concentrations (Pinckney et al. 1998) and the use of CHEMTAX (Mackey et al. 1997, Wright et al. 1996). Briefly, filters containing phytopigments were sonicated in 100% acetone (3 ml) for 30 seconds and then extracted in the dark for 20-24 h at -20° C. Extracts were filtered (0.2 µm) and injected (300 ul) into an HPLC system equipped with reverse-phase C_{18} columns in series (Rainin Microsorb-MV, 0.46 x 10 cm, 3 mm, Vydac 201TP, 0.46 x 25 cm, 5 mm). A nonlinear binary gradient, adapted from Van Heukelem et al. (1994), was used for pigment separations. Solvent A consisted of 80% methanol and 20% ammonium acetate (0.5M adjusted to pH 7.2), and Solvent B was 80% methanol and 20% acetone. Absorption spectra and chromatograms were acquired using a Shimadzu SPD-M10av photodiode array detector, where pigment peaks were quantified at 440 nm.

Using the measured phytopigment concentrations, biomasses of higher phytoplankton taxa were estimated with CHEMTAX. CHEMTAX is a matrix factorization program that enables the user to estimate the abundances of higher taxonomic groups from concentrations of pigment biomarkers (Mackey et al. 1997, Wright et al. 1996). The program uses a steepest descent algorithm to determine the “best fit” of an unknown sample to an initial estimate of pigment ratios for targeted algal taxa. The taxa used in the analysis were cyanobacteria, euglenophytes, chlorophytes, prymnesiophytes, cryptophytes and diatoms, which were selected because of their prevalence in Lake Granbury at the time of the experiments.

Sampling also included enumeration of zooplankton. Zooplankton were collected using a 12-liter Schindler trap, concentrated to 50 ml and preserved in 2.5% buffered formalin. Subsamples, 10-15 ml, were settled for a 24-hr period, then counted using an inverted, phase-contrast, light-microscope (40x and 200x, Leica Microsystem Inc.). For each individual counted, geometric shapes were determined that best corresponded to the shape of the zooplankter and dimensions were measured that enabled calculation of the individual's biovolume (Wetzel and Likens 1991). Identification was to the taxonomic level of genus. For purposes of this report, zooplankton were categorized into total copepod adults, copepod nauplii, total rotifers, and protozoa. The enumeration technique typically resulted in ~200 individuals counted per sample.

Samples for inorganic nutrients (nitrogen and phosphorus) were filtered through pre-combusted GF/F filters, and the filtrates were frozen until analysis. Inorganic nutrient concentrations were determined using autoanalyzer methodology (Armstrong and Sterns 1967, Harwood and Kuhn 1970). For this study, nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4) were summed as dissolved inorganic nitrogen (DIN), and phosphorus was soluble reactive phosphorus (SRP).

Salinity, temperature, and pH were determined with a water quality multi-probe (Quanta, Hydrolab) and light penetration was determined with a Secchi disk.

Since toxins produced by *P. parvum* under various physiological states are not fully understood, standards for measuring concentrations of toxins are not available at this time. Toxicity was estimated, however, using other methods. In previous studies, researchers commonly employed an in vitro hemolytic assay (Johannson and Granéli 1999, Barriero et al. 2005, Uronen et al. 2005) or non-standardized in vivo bioassays to assess biological effects of *P. parvum* cultures under nutrient limitation. In this study, ambient toxicity from each enclosure and field sample was evaluated rigorously using a standardized 24-hr static acute toxicity assay with the fathead minnow (*P. promelas*) model and a standardized 10-day static renewal chronic toxicity test with a cladoceran (*D. magna*) model, generally following standardized aquatic toxicology methodology (US EPA 1994, 2002), as reported previously (Roelke et al. 2007, Brooks et al. 2010).

To evaluate toxicity relationships among treatment combinations, ambient samples were diluted using a 0.5 dilution series with reconstituted hard water (RHW), which was performed according to US EPA recommendations (US EPA 2002). This dilution approach is routinely used to evaluate water quality of surface waters because it allows for assessment of relative extracellular toxicity among samples if an undiluted ambient sample is acutely toxic.

For each *P. promelas* toxicity test sample from each experimental unit and field sample, three replicate chambers with seven organisms per chamber were used to assess toxicity at each dilution level. *D. magna* bioassays followed established US EPA protocols (US EPA 1994). RHW, prepared according to standard methods (APHA 1998), was used as control treatment water for all toxicity assays. Alkalinity (mg/L as $CaCO_3$) and hardness (mg/L as $CaCO_3$) of RHW was measured potentiometrically and by colorimetric titration, respectively, before initiation of acute studies (APHA 1998). Specific conductance (µS/cm), pH, and dissolved oxygen (mg/L) of RHW was also measured before toxicity testing. All toxicity tests were performed in climate-controlled chambers at 25 ±1°C with a 16:8 hour light-dark cycle. Less than 48-hr-old fathead minnow larvae were fed newly hatched *Artemia* nauplii 2 hr before initiation of testing (US EPA 2002). *D. magna* were fed a Cerophyll®/green algae suspension daily, which was prepared according to methods reported previously (Brooks et al. 2004, Dzialowski et al. 2006). LC50 values for fathead minnow toxicity tests were estimated as percentage of ambient sample using Probit (Finney 1971) or Trimmed Spearman Karber (Hamilton et al. 1977) techniques, as appropriate.

Differences in response variables between experimental treatments were tested for significance using various general linear models (GLM, SPSS Inc.). For most variables, repeated-measures analysis of variance (ANOVA), using Wilk's multivariate F-test, was employed. To dissect treatment effects at different times during the experiment, univariate ANOVA was used, followed by Tukey's HSD test. Twelve enclosures were used as controls in these analyses (three planned controls plus nine untreated enclosures intended for other treatments that were not employed). Therefore, the HSD tests were adjusted for these differences in degrees of freedom. In the first experiment during bloom initiation, samples for testing acute toxicity to fish were not taken from the nine untreated enclosures, so only three control enclosures were used in statistical analyses, and because nearly all

enclosures were not detectably toxic on days 7 and 14, acute toxicity to fish was statistically analyzed only for day 21. In the second experiment during bloom development and decline, data on acute toxicity to fish were analyzed for days 7, 14, and 21 using repeated measures ANOVA. For this experiment, acute toxicity to fish was tested on all 12 control enclosures. For both experiments, non-toxic cultures were coded to LC50 of 100%, and LC50 data were analyzed on a percent dilution basis. Some response variables appeared to have heteroscedasticity, and were log-transformed and reanalyzed statistically, but because results were nearly always the same as results for raw data, only the latter are reported here.

2 Technical Reporting and Discussion

Lake conditions (extended record)

For the monitoring period, inflows into Lake Granbury were episodic, as is common in lakes of the south-central United States. From September 2006 through March 2007, inflows were barely discernible (Figure 3). In April 2007, a large inflow event occurred, with peak flows attaining 80 x 10^6 m^3 d^{-1}. Episodic inflows of varying magnitude and duration persisted through June, where the largest inflow event reached ~120 x 10^6 m^3 d^{-1}. The lake then entered a period of low inflows, lasting until after the initiation of the second in-lake experiment in 2010.

According to pigment analyses, phytoplankton biomass peaked in March 2007 just prior to the first large inflow event of 2007, with highest biomass occurring towards the lower end of the lake (Figure 3a). The next prominent peak in phytoplankton biomass occurred in late 2009, where chlorophyll *a* concentrations reached ~90 μg $liter^{-1}$. During the period of the in-lake experiments in 2010, chlorophyll *a* throughout the lake was in the range of 10-20 μg $liter^{-1}$.

Nearly coincident with the first chlorophyll *a* concentration maximum, a system-wide *P. parvum* bloom reached its highest population densities of ~40 x 10^6 cells $liter^{-1}$ in February 2007 with highest population densities occurring in the mid-reaches of the lake (91% of the phytoplankton biovolume). Cell densities >10 x 10^6 cells $liter^{-1}$ are considered bloom proportions based on historical observations in lakes of the south-central United States (Roelke et al. 2007, Schwierzke et al. 2010). Average *P. parvum* densities for the lake declined ~27% by March 2007 (Figure 3b). Measurements of ambient toxicity to fish were consistent with observed population densities, with LC_{50} values as low as 4% observed in February in the mid reaches of the lake, with toxicity to fish decreasing (LC_{50} increasing) by March (see Roelke et al. (2010a)). *P. parvum* population densities for the lake were obliterated after the first large inflow event to the lake in 2007, decreasing by 89% from the March to April sampling. In addition, waters were no longer toxic to fish. With the exception of the low-density bloom at the end of 2008, *P. parvum* blooms were not system-wide for the remainder of this data record. Instead, blooms were localized and occurring at different times throughout the lake. During the

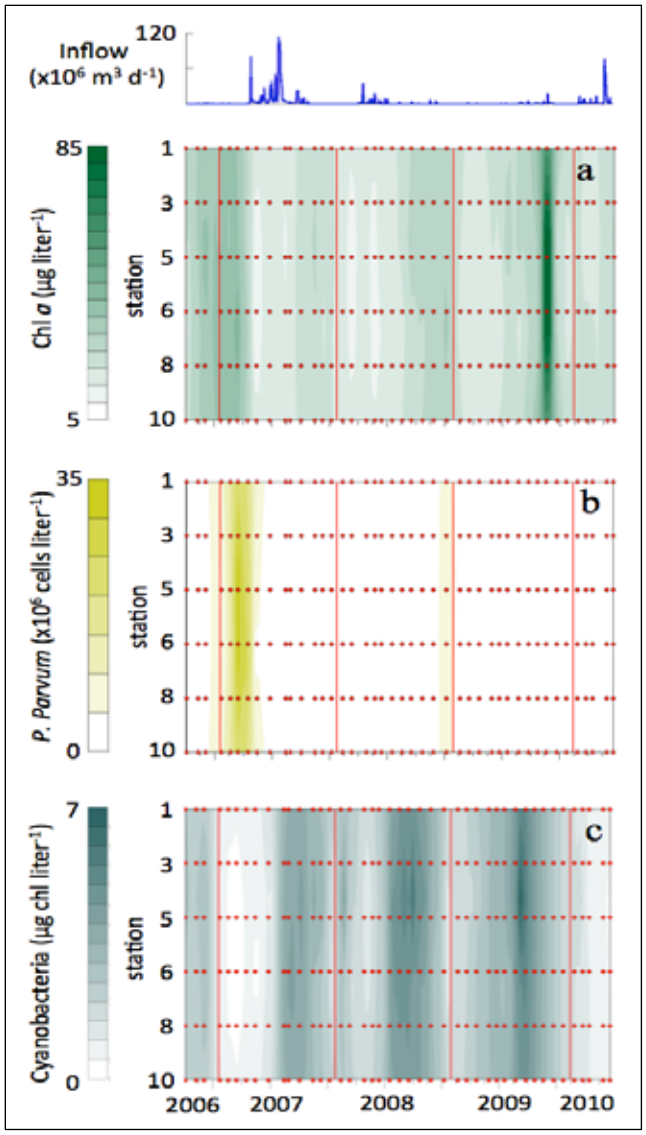

Figure 3. Time series data of chlorophyll a (a), *P. parvum* population density (b), and cyanobacteria biomass (c) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling stations encompassed the entire length of the lake, with station 10 being at the dam.

2010 period of study, *P. parvum* population densities remained below the defined bloom level of 10 x10^6 cells liter^{-1} throughout much of the lake.

Cyanobacteria were not abundant in Lake Granbury during the time of bloom development or termination in 2006-2007, but maximal from July through September throughout this period of study (Figure 3c). During the 2010 period when the in-lake experiments were conducted, cyanobacteria were lower, in the range of 1-1.5 µg-chl *a* liter^{-1}.

Dissolved inorganic nutrients sometimes showed a strong relationship with inflow. Immediately following the first large inflow event in April 2007, both DIN and SRP reached maxima of ~24 µM-N and ~0.55 µM-P (Figures 4a and 4b). Highest nutrient concentrations were measured in the lower reaches of the lake at this time. During January through March 2007, when *P. parvum* population densities were greatest and then started to decline, the DIN:SRP was ~30 with DIN concentrations ~1.35 µM-N and SRP ~0.05 µM-P. Except for December 2006, nutrient concentrations during the bloom of 2006-07 were similar to the months prior to the bloom. In December, SRP concentrations were at their lowest,

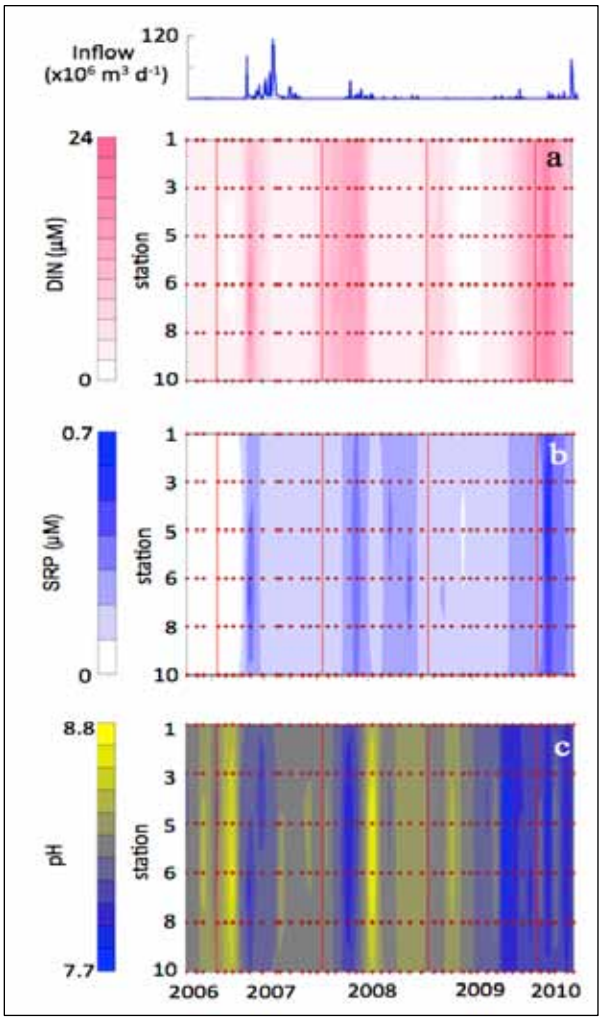

Figure 4. Time series data of dissolved inorganic nitrogen (a), soluble reactive phosphorus (b), and pH (c) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling stations encompassed the entire length of the lake, with station 1 being the lake's headwaters and station 10 being at the dam.

~0.03 μM-P, while DIN was ~3.55 μM-N (DIN:SRP ~122).Inorganic nutrient maxima were present during 2008, but they did not always correspond with inflows. Inorganic nutrient maxima were not apparent during 2009, nor were significant inflow events. During 2010, and at the time of the in-lake experiments, inorganic nutrient concentrations were relatively high in the lake.

While pH varied spatially and temporally, a prominent feature was relatively high pH coinciding with the *P. parvum* bloom in 2006-07, while a rapid decrease in pH immediately followed the first large inflow event in April 2007 (Figure 4c). Large fluctuations in pH were apparent in the lake for the entire period of study.

As with pH, zooplankton populations varied spatially and temporally. Peak population densities, however, occurred later in the period of study when system-wide *P. parvum* blooms did not occur (Figure 5).

Cove conditions [spanning the period of in-lake experiments]

Phytoplankton biomass accumulated in the cove during the 2010 period of in-lake experiments. At the start of the first experiment, on February 23, chlorophyll *a* was 13.5 μg liter^{-1}, while at the start of the second experiment, March 31, it was 22.9 μg liter^{-1} (Figure 7a). The population of *P. parvum* mirrored this trend, with densities of 0.76 and 1.1 x10^{6} cells liter^{-1} (Figure 7b). Toxicity in the cove was only observed on April 13, with an LC50 of 77% dilution (not shown), which occurred after the start of the second experiment.

Inorganic nutrients showed an opposite trend with phytoplankton biomass. At the start of the first experiment, DIN and SRP were 50 μM and 0.6 μM (N:P ~83, suggesting P-limiting conditions), while at the start of the second experiment, DIN and SRP were 0.5 μM and 0.1 μM (N:P ~5, suggesting N-limiting conditions) (Figures 8a and 8b). The range over which pH varied during this period was small, with values of ~7.8 and ~7.7 at the start of the first and second experiments (Figure 8c).

Zooplankton showed taxa-specific trends in the cove during the period of in-lake experiments. For example, copepod adults and nauplii increased in density, with populations nearly tripling from the start of the first experiment compared to the second (Figures 9a and 9b). Rotifers increased nearly 5-fold at these times (Figure 9d). Cladocerans, on the other hand,

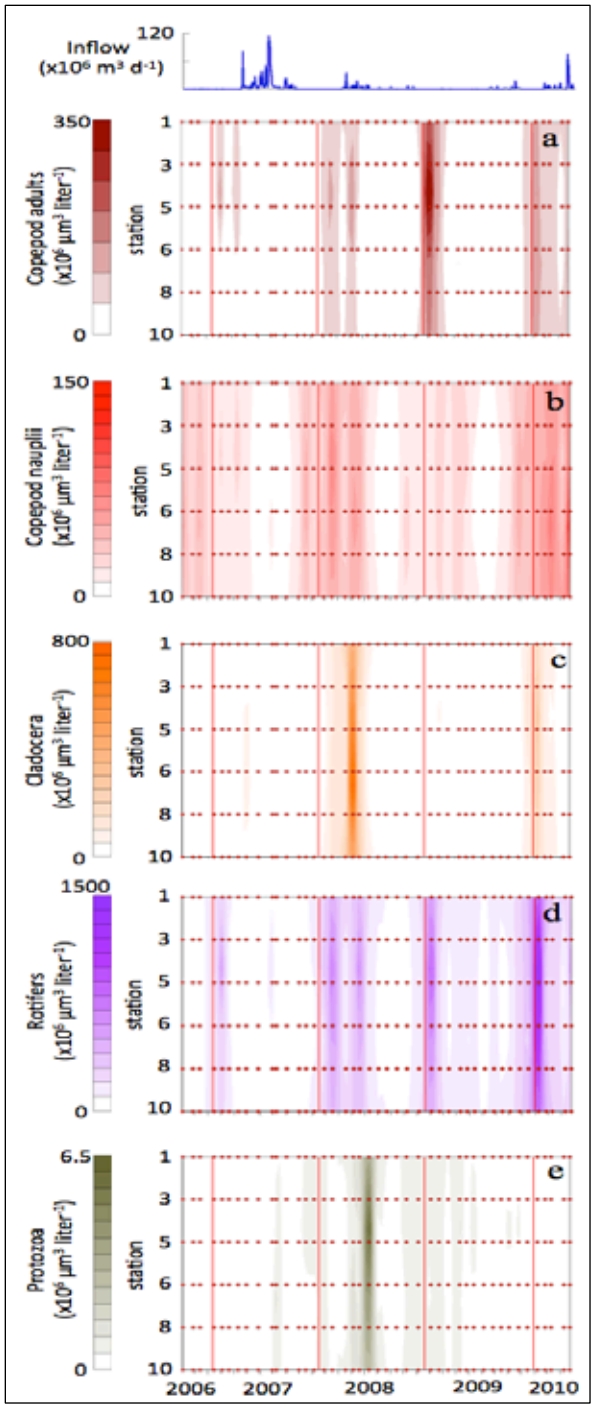

Figure 5. Time series data of zooplankton, which include adult copepods (a), copepod nauplii (b), cladocerans (c), rotifers (d), and protozoa (e) from monthly sampling trips performed in Lake Granbury where trips spanned five years and locations of sampling station encompassed the entire length of the lake, with station 1 being the lake's headwaters and station 10 being at the dam.

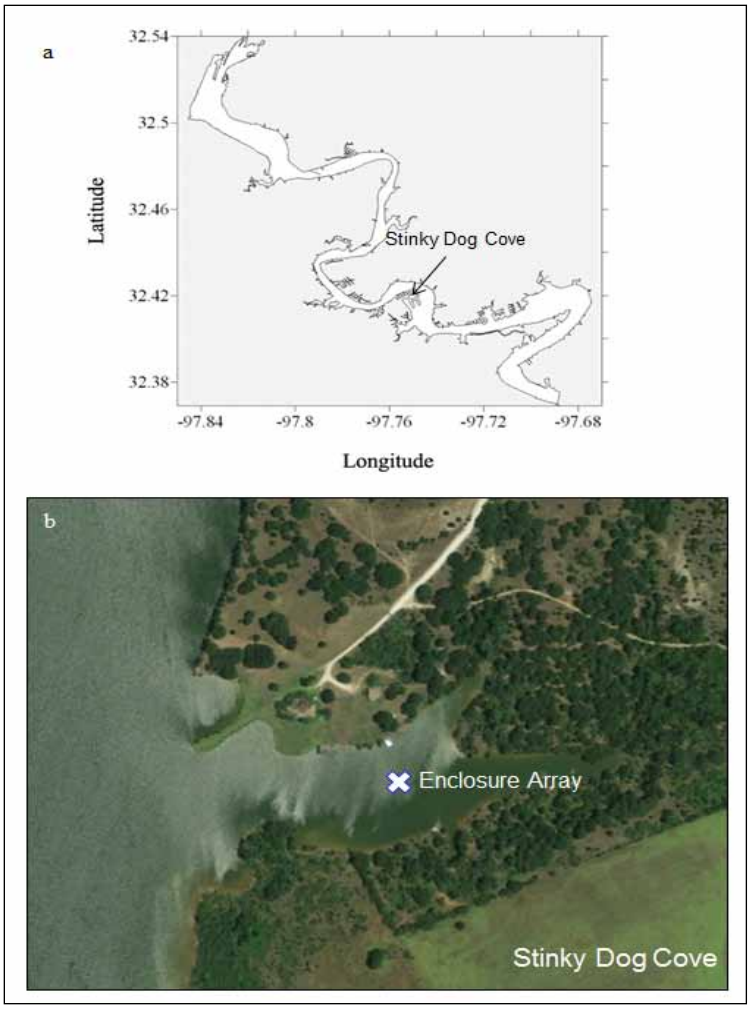

Figure 6. Location of Stinky Dog Cove, Lake Granbury, TX, where the enclosure experiments were performed.

decreased ~50% from the start of the first experiment compared to the second (Figure 9c). Protozoans showed a population maxima during the period of in-lake experiments (Figure 9e), but accumulation of this population did not commence until after the beginning of the first experiment, and its decline was not complete before the second experiment was begun.

In-lake experiments

Because the first experiment commenced before significant accumulation of *P. parvum* cells in the cove and because water conditions had not yet become toxic, this period is referred to as "pre-bloom" in the text below. The period when the second experiment commenced will be referred to as "bloom development and decline" because *P. parvum* population density was higher in the cove and toxicity was observed.

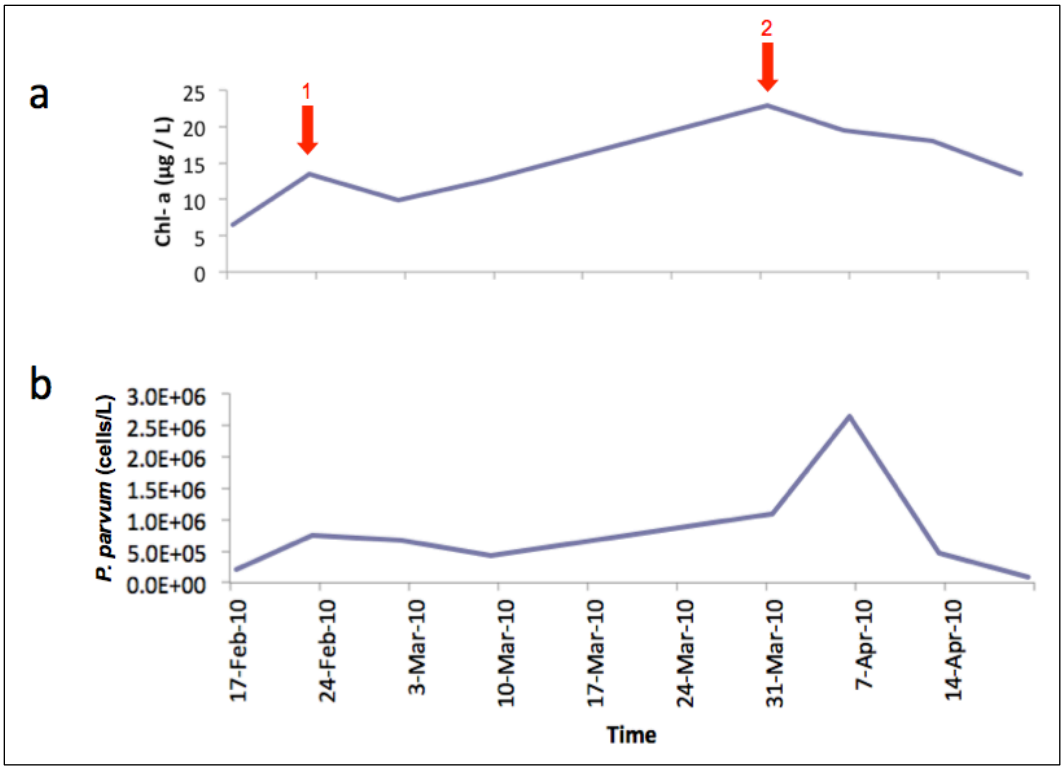

Figure 7. Time series data of chlorophyll *a* (a) and *P. parvum* population density (b) from weekly sampling trips performed in Lake Granbury where samples were collected in the cove in which the experiments were deployed. Arrows indicate the start dates of the experiments.

Statistical analysis

For most response variables in each experiment, treatment effects over the duration of the experiment were tested with a repeated-measures (RM) analysis of variance (ANOVA), using Wilk's multivariate F-test. For the flushing treatments, a one-way analysis was used for both experiments with four groups: control, low flushing (0.05 d^{-1}), intermediate flushing (0.1 d^{-1}) and high flushing (0.3 d^{-1}). For the pH manipulations, a one-way analysis was used with three groups for the first experiment: control (~8.5 during the experiment) and two lowered pH levels (7.5 and 7.0); and four groups for the second experiment: control (again, ~8.4) and three lowered pH levels (8.0, 7.5, 7.0). For the NH_4 additions, a one-way analysis was used for both experiments with three groups: controls, low NH_4 additions (10 µM), and high NH_4 additions (40 µM). To dissect treatment effects at different times during the experiment, univariate ANOVA was used, followed by Tukey's HSD test.

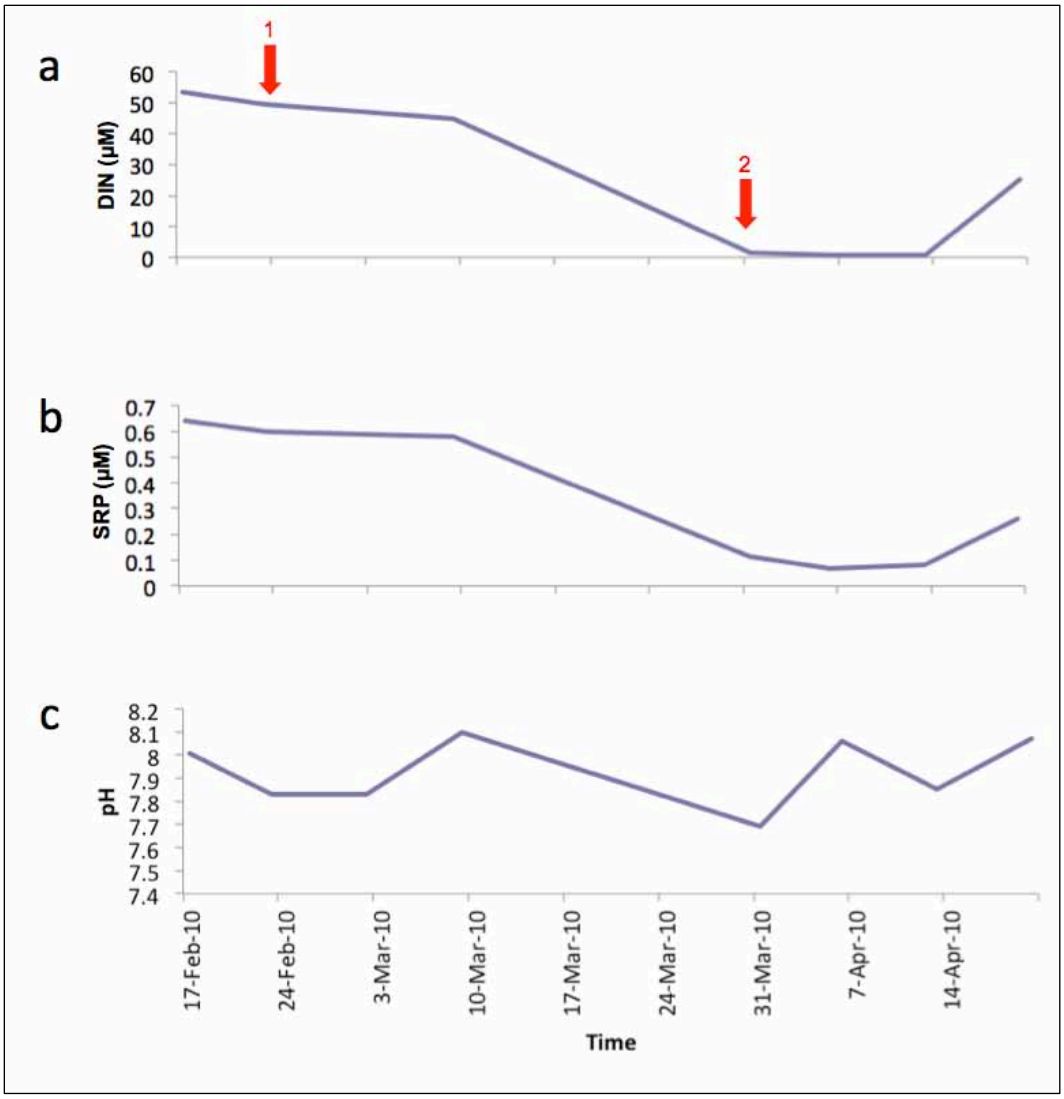

Figure 8. Time series data for dissolved inorganic nitrogen (a), soluble reactive phosphorus (b), and pH (c) from weekly sampling trips performed at Lake Granbury in the cove where the experiments were deployed. Arrows indicate the start dates of the experiments.

A total of 12 enclosures were used as controls in these analyses (three planned controls plus nine untreated enclosures intended for other purposes). Therefore, the HSD tests were adjusted for these differences in degrees of freedom. In the first experiment during bloom initiation, samples for testing acute toxicity to fish were not taken from the nine untreated enclosures, so only three control enclosures were used in statistical analyses, and because nearly all enclosures were not detectably toxic on days 7 and 14, acute toxicity to fish was statistically analyzed only for day 21.In the second experiment during bloom development and decline, data on acute toxicity to fish were analyzed for days 7, 14, and 21 using RM-ANOVA. For this experiment, acute toxicity to fish was tested

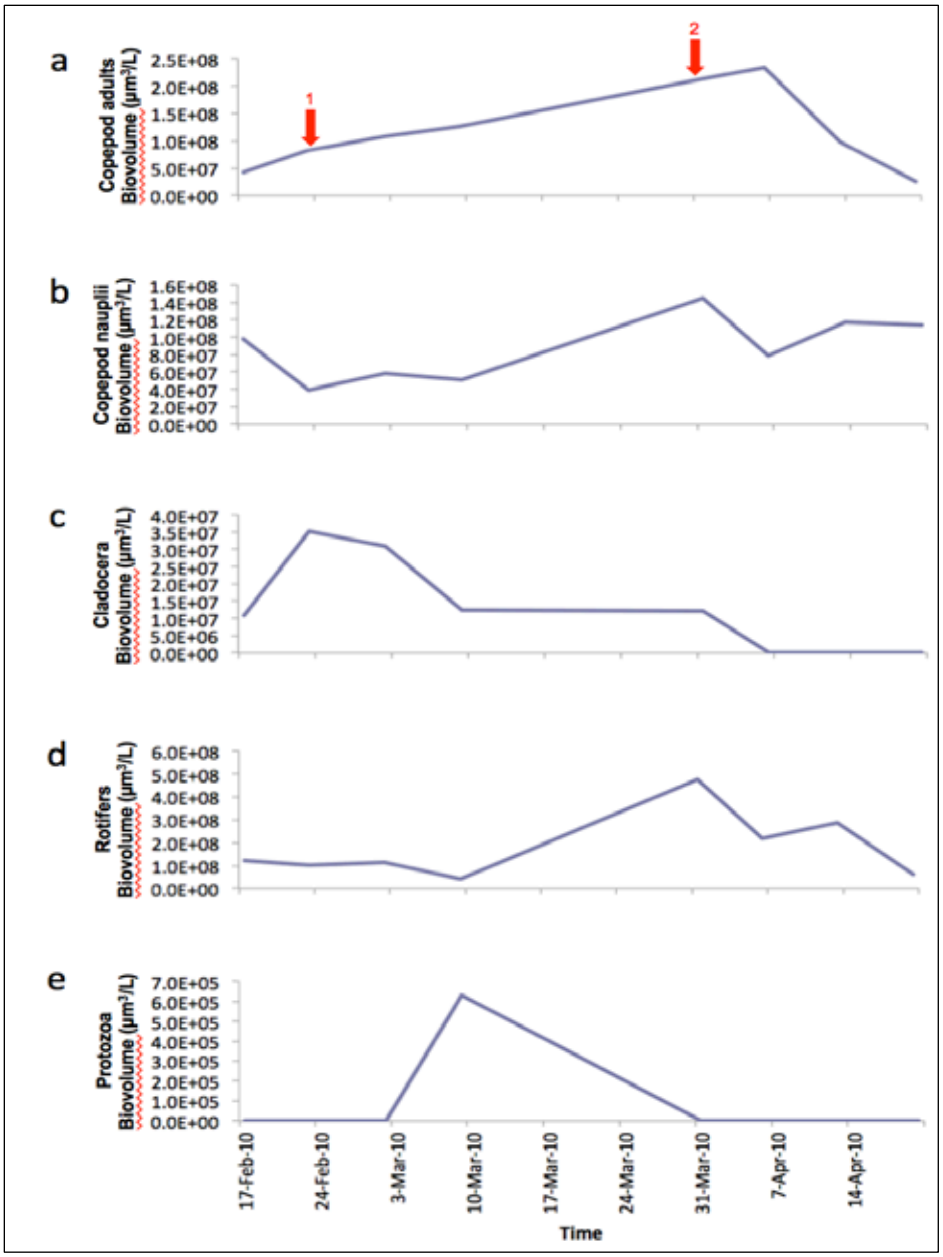

Figure 9. Time series data of zooplankton, which include adult copepods (a), copepod nauplii (b), cladocerans (c), rotifers (d), and protozoa (e) from weekly sampling trips performed in Lake Granbury where samples were collected in the experimental cove. Arrows indicate the start dates of the experiments.

on all 12 control enclosures. For both experiments, non-toxic cultures were coded to LC50 of 100%, and LC50 data were analyzed on a percent dilution basis. For both experiments, effects on Daphnia reproduction were tested only on day 21, so univariate ANOVA was used. Three control cultures were tested in the first experiment, and 12 in the second experiment. Some response variables appeared to have heteroscedasticity, and were log-transformed and reanalyzed statistically, but because results

were nearly always the same as results for raw data, only the latter are reported here.

First experiment – Pre-bloom conditions

Control

P. parvum population density was well below defined bloom levels at the start of the pre-bloom experiment (Figure 10). In addition, water was not toxic to fish (Figure 11). As the experiment progressed, however, *P. parvum* accumulated, eventually exceeding bloom levels by the end of the experiment with waters becoming toxic. Total phytoplankton biomass peaked at T7 (Figure 12), with zooplankton biomass peaking at T14 (Figure 13). Both decreased as *P. parvum* accumulated and water became toxic.

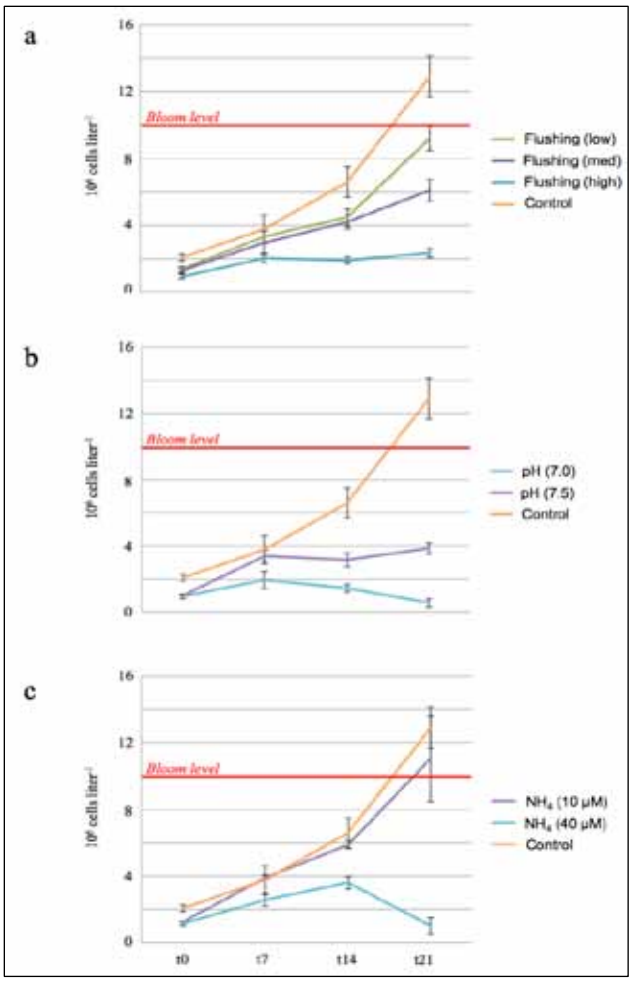

Figure 10. Time series data of *P. parvum* population density from weekly samplings of the pre-bloom experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

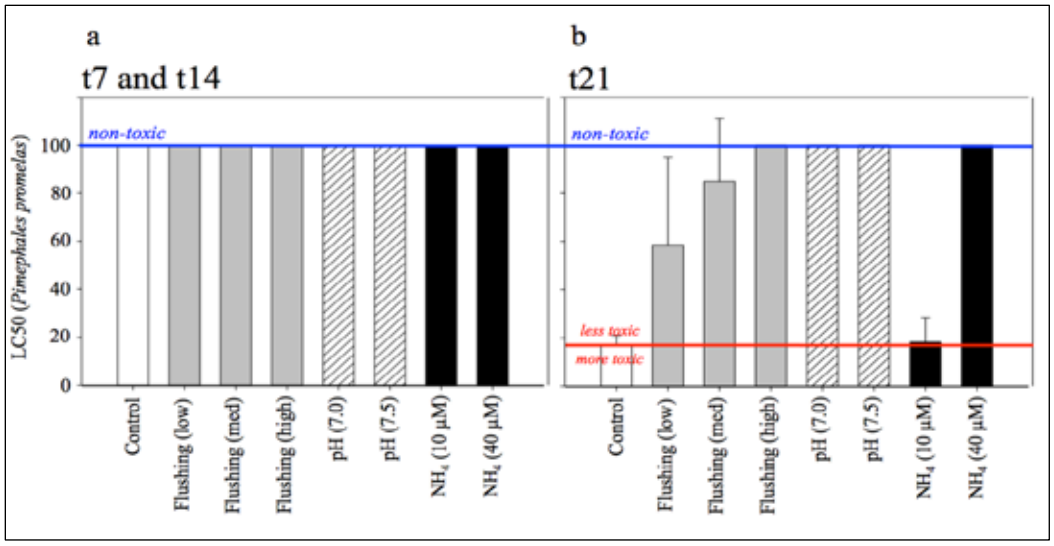

Figure 11. Water toxicity data from the pre-bloom experimental treatments during the 7th and 14th day of the experiment (a) and at day 21 (b).

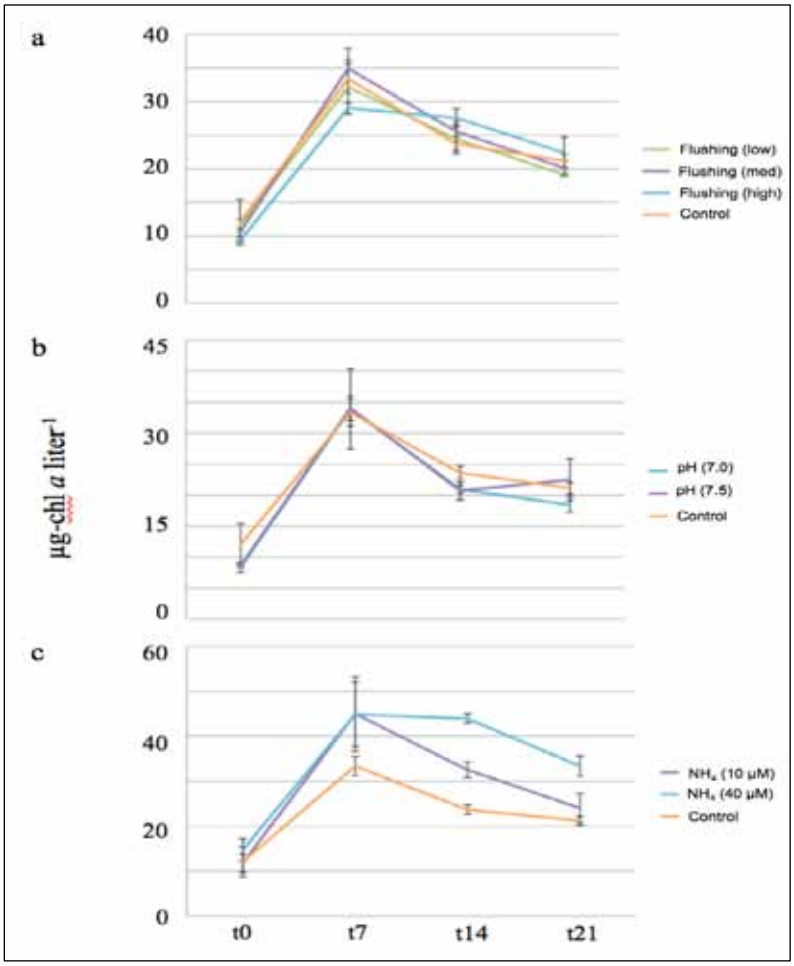

Figure 12. Time series data of total phytoplankton biomass from weekly samplings of the pre-bloom experiment from enclosures that were the flushing (a), pH (b), and NH4 (c) treatments.

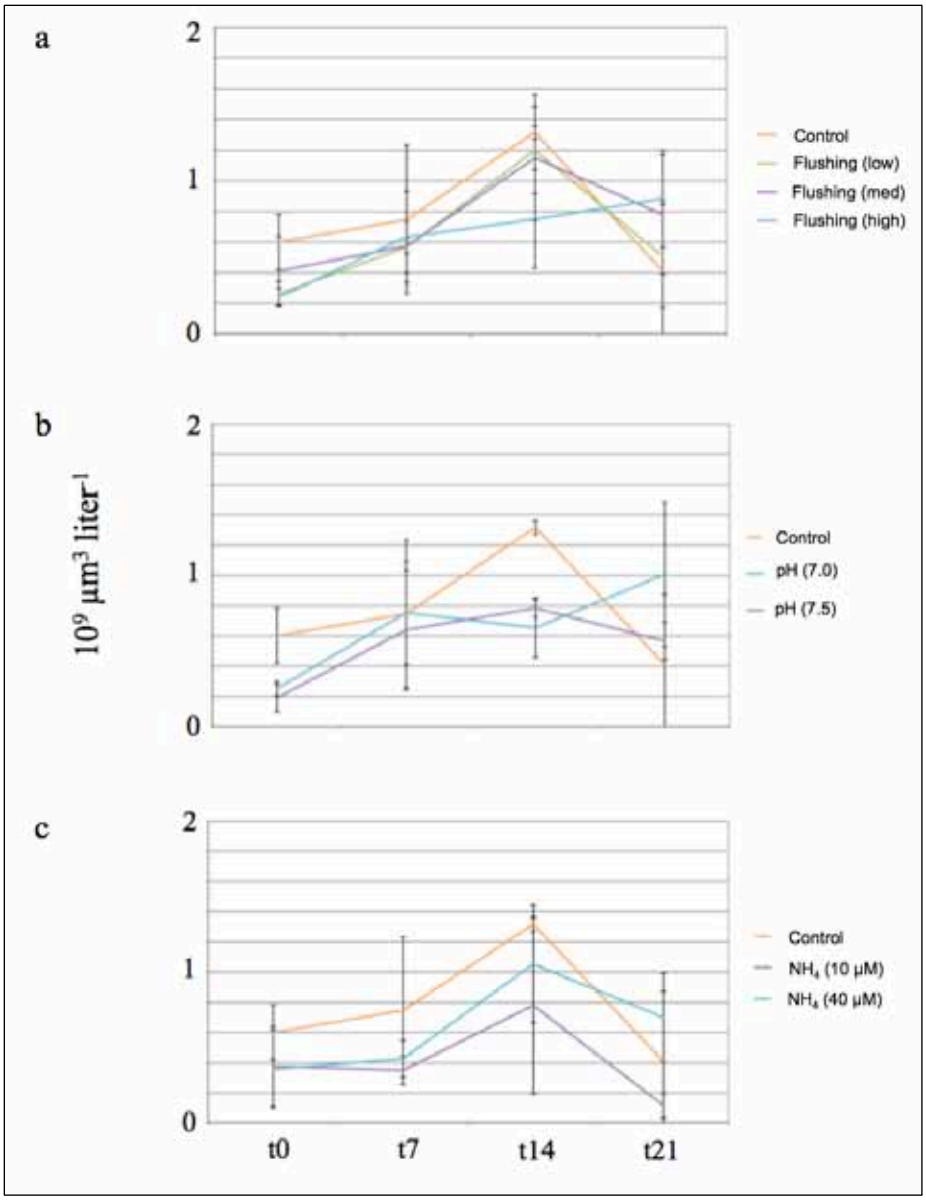

Figure 13. Time series data of total zooplankton biomass from weekly samplings of the pre-bloom experiment from enclosures that were the flushing (a), pH (b), and NH4 (c) treatments.

Flushing

Flushing treatments during the pre-bloom experiment slowed the accumulation of *P. parvum* cells and the exchange magnitude had a strong negative correlation with *P. parvum* density. High magnitude flushing resulted in significantly lower *P. parvum* density (*P*-value<0.001). However, low flushing treatments reached higher population densities than the control, although this difference was not statistically significant. Similarly, medium treatments had a weak effect, in that *P. parvum* densities

alternated between higher and lower concentrations compared to the control; again, these minor differences were not statistically significant (Figure 8a).

Ambient toxicity was subsequently reduced by source water flushing. All mesocosms were non-toxic from initiation through T14 (Figure 11a); however, by the end of the experiment (T21), conditions in the control mesocosms had become extremely toxic (Figure 11b). Results from LC_{50} tests represented the acute toxicity to juvenile fathead minnows (*Pimephales promelas*) through % survivorship when exposed to sample water for 48 hr. Toxicity levels in the control mesocosms resulted in ~17% survivorship of minnows, low flushing treatments had ~59% survivorship, medium treatments ~85% and were significantly lower than the control (*P* –value=0.024), and high level flushing resulted in 100% survivorship with no deviation in any of our replications (*P* –value=0.008).

Phytoplankton biomass increased during the pre-bloom experiment, peaking at T7, then decreasing for the remainder of the experiment (Figure 12a). Flushing had no observable effect.

Zooplankton biomass increased during the pre-bloom experiment and peaked at T14, following an increase in phytoplankton, then decreased with their phytoplankton prey by T21 (Figure 13a). High flushing had a dilution effect, slowing the accumulation of zooplankton, although statistically non-significant ($p > 0.05$).

pH manipulation

When the pre-bloom experiment was initiated on T0, there was no difference between cove pH and limnocorral pH values; however, by T21, pH values in control limnocorrals were increased (mean pH = 8.86; n = 3) relative to the cove pH (8.1). As noted above, at T0, T7, and T14 during pre-bloom experiment 1, *P. parvum* cells were present in all of the limnocorrals, and increased in density in the control experimental units. By T21, *P. parvum* densities in both pH treatment levels (7, 7.5) were significantly reduced (ANOVA; $p < 0.05$) relative to controls, which exceeded harmful algal bloom (HAB) threshold levels (Figure 10).

Though an HAB had not formed and limnocorral samples were not acutely toxic to *P. promelas* (Figure 11a) or sublethally toxic to *D. magna* at T7 or

T14, a highly toxic HAB to *P. promelas* had formed in control limnocorrals by T21 (Figure 11b). pH treatment levels (7, 7.5) were highly effective at ameliorating ambient toxicity (ANOVA; $p<0.05$); no *P. promelas* mortality was observed throughout the study in limnocorrals reduced to pH 7 or 7.5 (Figures 11a and 11b).

As noted above, phytoplankton biomass increased during the pre-bloom experiment 1, peaked at T7, then decreased for the remainder of the experiment (Figure 12b). However, pH treatment levels (7, 7.5) were not significantly different from controls ($p>0.05$).

Similarly, zooplankton biomass increased during the pre-bloom experiment, peaked at T14 in response to an increase in phytoplankton biomass, but decreased by T21 (Figure 13b). Similar to the phytoplankton biomass response variable, no pH effects were observed (Figure 13b).

NH_4 addition

Ammonium additions were intended to raise dissolved NH_4 concentrations by 10 and 40 μM. Determinations of dissolved NH_4 suggest that concentrations higher than these nominal levels were obtained. On day 0, immediately after treatment, NH_4 averaged 16.0 μM in low NH_4 enclosures, and 88.1 μM in high NH_4 enclosures (Figure A2). Accounting for NH_4 in control enclosures on day 0 (average of 8.0 μM), about 85% of the intended level was obtained in the low NH_4 addition enclosures, and 265% in the high NH4 addition enclosures. Although it is possible that enclosure volumes were smaller than intended, it is also possible that high NH_4 in samples taken shortly after NH_4 additions was a result of incomplete mixing to the depth of the enclosures. Dissolved NH_4 increased during the experiment, reaching an average of 38.2 μM in enclosures with low NH_4 additions, and an average of 121.1 μM in enclosures with high NH_4 additions. These concentrations are 127% and 101% of what would be achieved if NH_4 treatments were perfectly mixed and accumulated conservatively over the course of the experiment. Ammonium concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 351.65$, $P < 0.001$) on all days of the experiment (ANOVA, $P < 0.05$), and significantly higher on average for low NH_4 additions than controls, and significantly higher still for high NH_4 additions (Tukey's HSD, $P < 0.05$).

Temperature and pH were virtually identical in enclosures with low and high NH_4 additions. Temperature increased from about 7° C for days 0 – 14 to about 11° C on day 21, and in all enclosures, pH rose from initial values of about 8, to values of 8.8–9.0 on days 14 and 21 (Figure A4). Given the similarity of temperature and pH in the enclosures, calculated percentages of unionized NH_4 differed negligibly between enclosures with high and low NH_4 additions. Values of pH were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 3.45$, $P = 0.009$), but only on day 21 (ANOVA, $P < 0.05$), when pH was significantly lower on average for high NH_4 additions than controls and low NH_4 additions (Tukey's HSD, $P < 0.05$). However, this difference was about 0.06 units and had a negligible impact on the calculated percentage of unionized ammonium (< 1%). Calculated percentages of unionized NH_4 were 1–3% for days 0 – 7, and 12–13% for days 14 – 21. Salinity in all enclosures averaged 0.43 g liter^{-1}, with no discernible time trend and no significant differences between treatments (RM-ANOVA, Wilk's $F_{8,24} = 1.03$, $P = 0.44$). Thus, NH_4 additions did not detectably increase salinity in enclosures.

Nitrate concentrations decreased during the experiment in control enclosures, from 26.1 to 0.1 μM on average (Figure A1). In enclosures with low NH_4 additions, nitrate decreased from 19.1 to 12.6 μM on average, while enclosures with high NH_4 additions showed no obvious trend with average nitrate ranging 17.8 to 20.6 μM. Nitrate concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 81.52$, $P < 0.001$) on days 7, 14, and 21 (ANOVA, $P < 0.05$). On day 7, nitrate was significantly higher on average for high NH_4 additions than controls, and on days 14 and 21 nitrate was significantly higher on average in high NH_4 additions than in low NH4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$). Since nitrate did not increase over time, nitrification of added NH_4 seems unlikely during this experiment. The reduced depletion of nitrate with high additions of NH_4 could be due to preferential microbial uptake of NH_4.

The abundance of *P. parvum* increased in all enclosures from day 0 to 14, though less rapidly in enclosures receiving high NH_4 additions (Figure 10). These increases continued until day 21 in control enclosures and those with low NH_4 additions, reaching bloom levels exceeding 10×10^6 cells liter^{-1}. The abundance of *P. parvum* declined from day 14 to 21, however, in enclosures with high NH_4 additions and never exceeded bloom levels. Abundances of *P. parvum* were significantly affected by treatments

(RM-ANOVA, Wilk's $F_{8,24}$ = 6.35, P < 0.001) on days 7 and 21 (ANOVA, P < 0.05). On day 7, the abundance of *P. parvum* was significantly lower on average for high NH_4 additions than for low NH_4 additions, and on day 21 the abundance of *P. parvum* was significantly lower on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, P < 0.05). Although the abundance of *P. parvum* was somewhat higher with low NH_4 additions than in controls, consistent with a weak fertilizing effect, this difference was not statistically significant.

No samples from any enclosures taken on days 7 or 14 displayed acute lethal toxicity to fish; on day 21 samples from enclosures with high NH_4 additions were also not acutely toxic to fish, but those from controls and enclosures with low NH_4 additions were toxic, with LC 50 ranging from about 10%-30% dilution (Figure 11). On day 21, LC50 values were significantly affected by treatments (ANOVA, $F_{2,6}$ = 167.38, P < 0.001). LC50 was significantly higher (less toxic) on average for high NH_4 additions than for low NH_4 additions or controls (Tukey's HSD, P < 0.05).

Chlorophyll *a* increased from day 0 to 7 in controls and enclosures with low NH_4 additions, and then decreased until day 21; the increase continued in enclosures with high NH_4 additions until day 14, followed by a decrease to day 21 (Figure 12). On days 14 and 21, chlorophyll *a* was higher on average in enclosures with high NH_4 additions than those with low NH_4 additions, which were higher than controls, consistent with a fertilization effect for total phytoplankton biomass. Chlorophyll *a* was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24}$ = 27.25, P < 0.001) on days 7 and 21 (ANOVA, P < 0.05). On day 14, chlorophyll *a* was significantly higher on average for high NH_4 additions than for low NH_4 additions, which were significantly higher than controls, and on day 21 chlorophyll *a* was significantly higher on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, P < 0.05). *Daphnia* reproduction tested with samples from day 21 averaged 51 neonates (SD = 15), with significant differences among treatments (ANOVA, $F_{2,6}$ = 2.23, P = 0.19).

The total abundance of zooplankton increased from days 0 to 14 in all enclosures, reaching about 800 individuals liter^{-1}, and then declined on day 21 to levels lower than those observed initially (Figure 13). Total abundance of zooplankton was not significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24}$ = 1.06, P = 0.42), nor did univariate tests detect significant treatment effects on any day (ANOVA, P > 0.05). The total biovolume of

zooplankton also increased from days 0 to 14 in all enclosures, and then declined on day 21. However, for enclosures with high NH_4 additions, total biovolume of zooplankton on day 21 remained above the initial level, and was higher than in controls or enclosures with low NH_4 additions, suggesting that the fertilization effect on total phytoplankton biomass might have propagated to zooplankton biomass, albeit weakly. Total biovolume of zooplankton was not significantly affected by treatments overall (RM-ANOVA, Wilk's $F_{8,24} = 1.06$, $P = 0.42$), but on day 21 univariate analysis detected a significant effect (ANOVA, $P = 0.027$). On day 21, total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in low NH_4 additions (Tukey's HSD, $P < 0.05$).

Second experiment – Bloom development and decline

Control

At the start of the bloom development and decline experiment, *P. parvum* population density was just shy of the defined bloom level (Figure 14) with water still being non-toxic to fish. As the second experiment progressed, *P. parvum* accumulated, eventually reaching a density two-fold greater than the defined bloom level with waters becoming very toxic (Figure 15). Total phytoplankton biomass declined for much of this experiment, with a slight recovery at T21 (Figure 16). Zooplankton biomass declined for the duration of this experiment (Figure 17).

Flushing

Consistent with the first experiment, flushing treatments during the bloom development and decline experiment slowed the accumulation of *P. parvum* cells and the exchange magnitude had a strong correlation with *P. parvum* density. All three flushing levels produced *P. parvum* densities significantly lower than the control (P –value≤0.003), and the high level flushing circumvented bloom proportions (10 x 10^6 cells L^{-1}) from being reached throughout the length of the experiment (Figure 14a).

Also consistent with the first experiment, ambient toxicity was reduced by source water flushing. The same monotonic relationship observed during the first experiment was also illustrated in the bloom development and decline experiment (Figure 15). All mesocosms had moderate toxicity through T7, resulting in ~50% survivorship of minnows (Figure 15a). Within the successive week (T14), control, low flushing, and medium

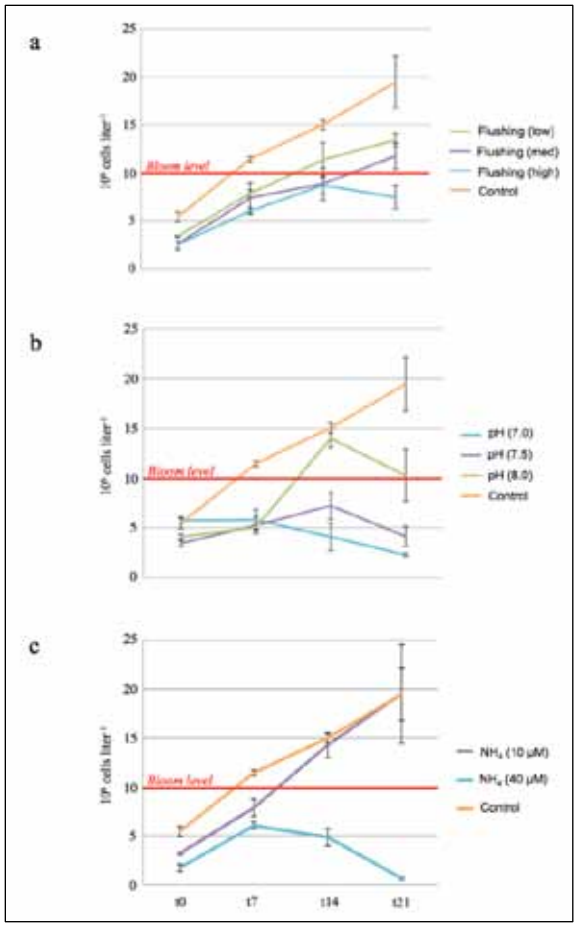

Figure 14. Time series data of *P. parvum* population density from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b), and NH4 (c) treatments.

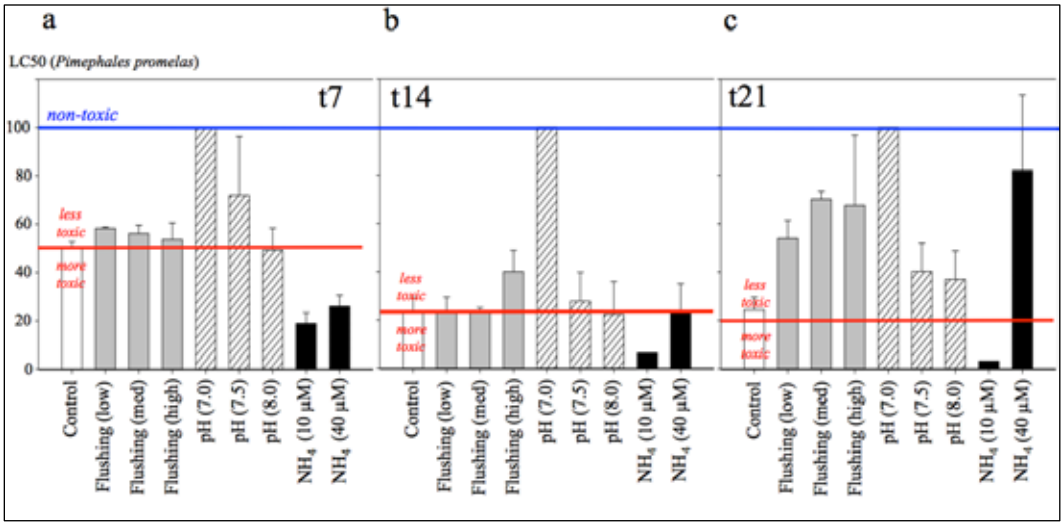

Figure 15. Water toxicity data from the bloom development and decline experimental treatments during the 7th (a), 14th (b), and 21st (c) day of the experiment.

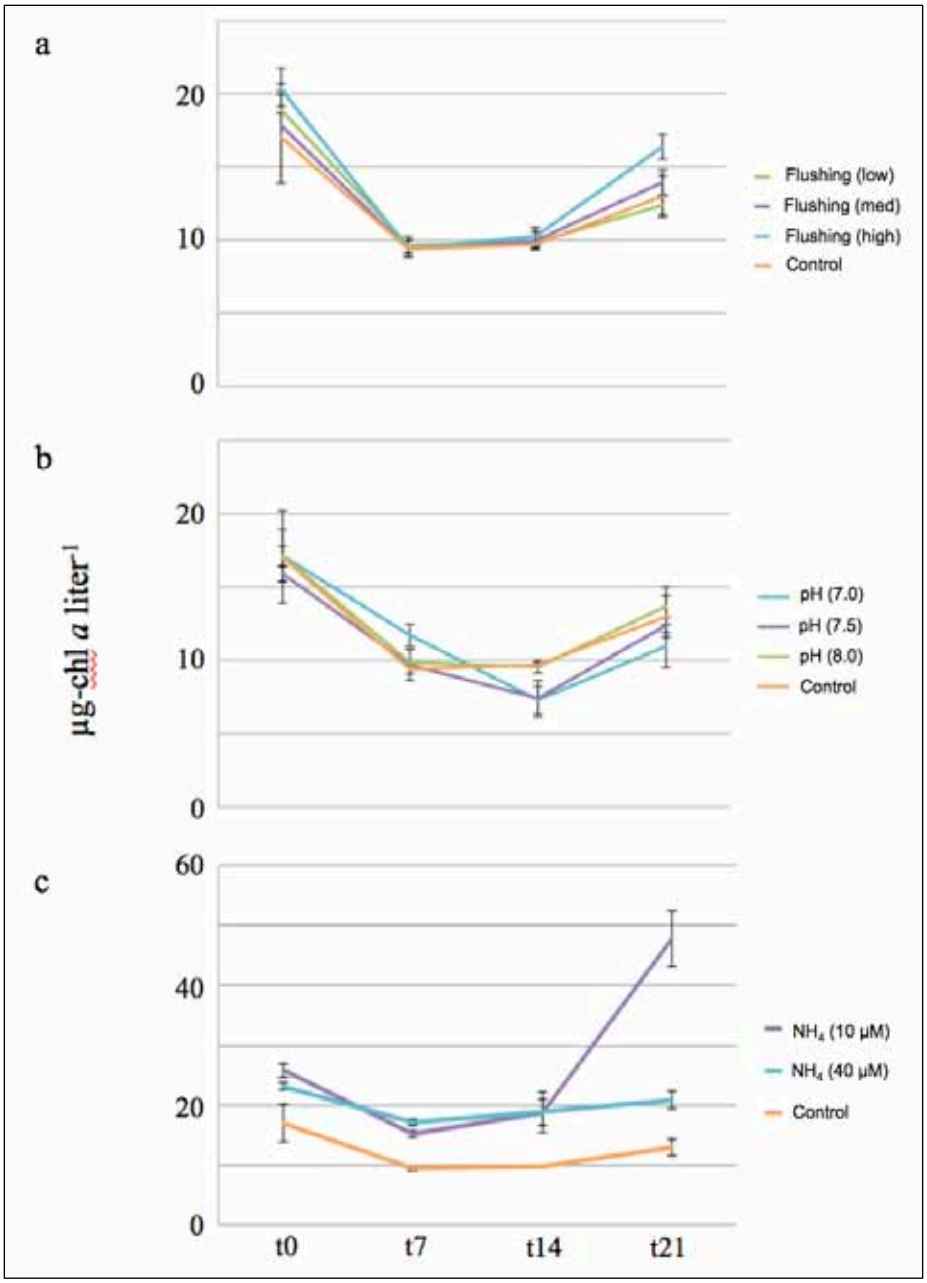

Figure 16. Time series data of total phytoplankton biomass from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b), and NH4 (c) treatments.

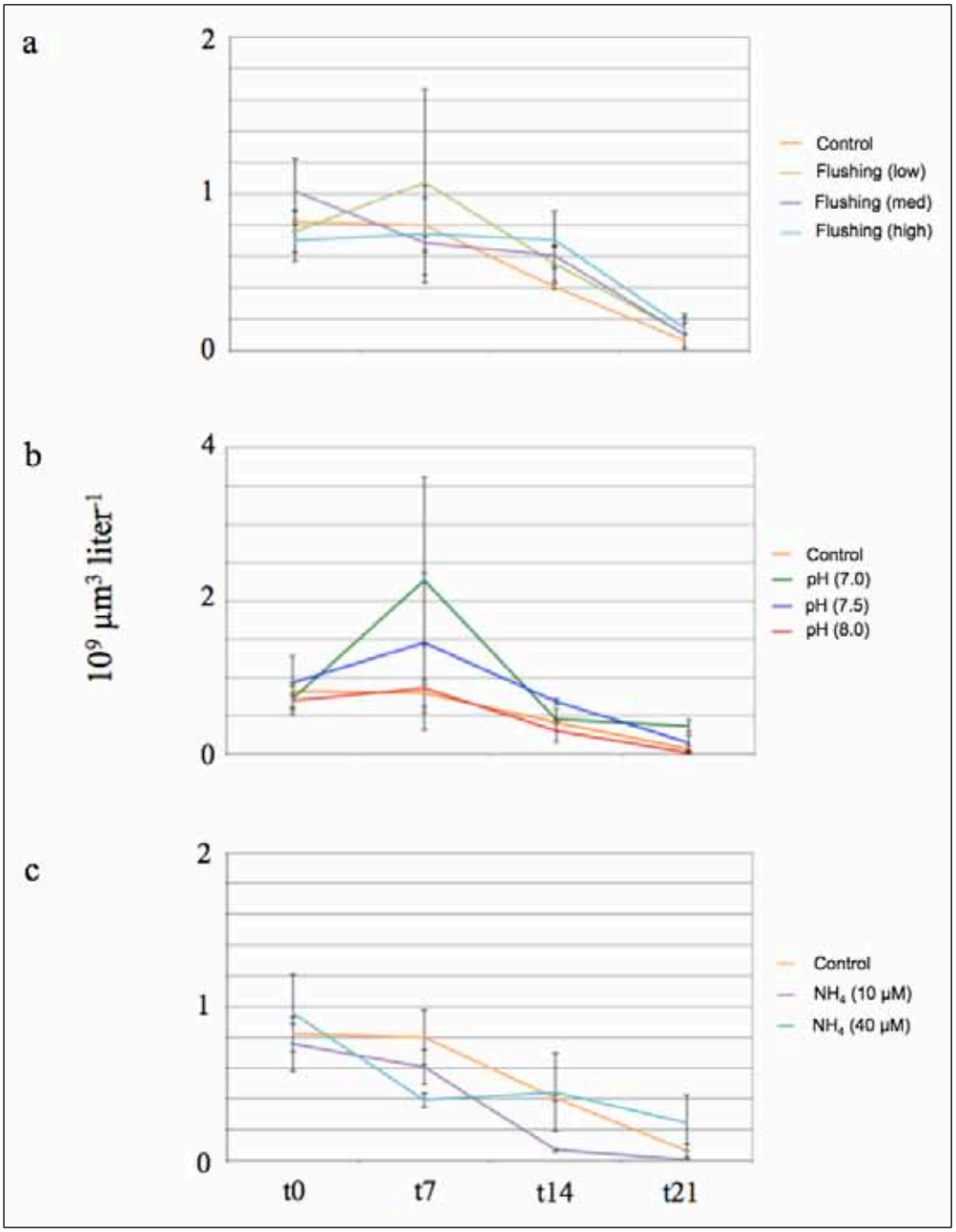

Figure 17. Time series data of total zooplankton biomass from weekly samplings of the bloom development and decline experiment from enclosures that encompassed the flushing (a), pH (b), and NH4 (c) treatments.

flushing conditions increased in toxicity at a similar rate, resulting in ~25% survivorship. High flushing treatment results diverged from this trend and exhibited a minimal increase in toxicity (~40% survivorship, Figure 15b). At the conclusion of the experiment (T21), both the high and medium treatments were significantly less toxic than the control (high flushing *P* –

value=0.004; medium flushing *P* –value=0.012). Control water yielded ~25% survivorship, the low flushing treatments decreased in toxicity to result in ~53% survival of fish, and both the medium and high flushing magnitudes had ~70% survival rate (Figure 15c).

Flushing had no observable effect on changes in total phytoplankton biomass, with chlorophyll *a* declining for much of this experiment and a slight recovery at T21 (Figure 16a).

Throughout the second experiment, zooplankton density was continually decreasing (Figure 17a). Slower rates of decline were witnessed in medium and high flushing treatments, especially at T14 and T21 when toxicity levels were significantly lower in these treatments and chlorophyll *a* levels were beginning to increase, representing grazing opportunities; however, these differences were statistically non-significant.

pH manipulation

Experiment 2 included an additional pH treatment level (pH = 8) to the experimental design. At T0, pH of control limnocorrals were raised to levels consistent with control conditions of experiment 1 (mean pH = 8.58; n = 3). As such, these levels were higher than ambient cove conditions (pH = 7.69 at T0) and represented the highest pH among the pH treatment levels (7, 7.5, 8). As noted above, when experiment 2 was initiated *P. parvum* cells were present in all limnocorrals at T0 and increased in density in the control experimental units. By T14 and continuing through T21, *P. parvum* densities in controls and the highest pH treatment level (8) exceeded HAB thresholds (Figure 14b), but lower treatment levels (7, 7.5) were significantly reduced ($p < 0.05$) relative to controls (Figure 14b).

Over the 21-day study, control limnocorrals became increasingly acutely toxic to *P. promelas* (Figure 15). Similar to experiment 1, a statistically significant difference was observed between *P. promelas* LC_{50} values and pH treatment level ($p<0.05$); Figure 15). Acute toxicity to fish was significantly ameliorated by the pH 7 treatment level, but not the 7.5 or 8 treatment levels, on T7, T14, and T21 (Figure 15). Though no statistically significant difference was observed between *D. magna* reproduction and pH treatments ($p=0.217$), mean reproduction increased from 16.2 neonates/female^{-1} in controls to 34.7 neonates/female^{-1} at pH 7, indicating a marked reduction of sublethal toxicity to cladocerans. A

statistically significant relationship was also observed between *P. parvum* cell density and both *P. promelas* LC_{50} (r^2= 0.47, p<0.05) values and *D. magna* reproduction (r^2=0.32, p<0.05).

As noted above, both phytoplankton biomass (Figure 16b) and zooplankton density (Figure 17b) continually decreased during experiment 2, but no significant effects of pH were observed on phytoplankton biomass and total zooplankton biomass.

Interestingly, on T21 cladocerans were only observed in two of the three low pH (7) limnocorrals, which corresponds to the observed decreased sublethal toxicity observed for *D. magna* reproduction responses at pH = 7 relative to controls and higher pH treatment levels.

NH_4

Determinations of dissolved NH_4 suggest that concentrations higher than the nominal levels of 10 and 40 μM were obtained. On day 0, immediately after treatment, NH_4 averaged 27.5 μM in low NH_4 enclosures, and 61.7 μM in high NH_4 enclosures (Figure A6). Accounting for NH_4 in control enclosures on day 0 (average of 1.0 μM), about 265% of the intended level was obtained in the low NH_4 addition enclosures, and 152% in the high NH_4 addition enclosures. Dissolved NH_4 increased during the experiment, reaching an average of 28.0 μM in enclosures with low NH_4 additions, and an average of 90.3 μM in enclosures with high NH_4 additions. These concentrations are 93% and 75% of what would be achieved if NH_4 treatments were perfectly mixed and accumulated conservatively over the course of the experiment. Although it is possible that enclosure volumes were smaller than intended, two other factors could have increased the measured NH_4 concentrations above expectations. Zooplankton abundance was high when the experiment was initiated, and declined in all enclosures (as detailed below), so it is possible that NH_4 was mineralized from dying zooplankton populations. It is also possible that high NH_4 in samples taken shortly after additions was a result of incomplete mixing to the depth of the enclosures. Ammonium concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24}$ = 2352.49, P < 0.001) on all days of the experiment (ANOVA, P < 0.05), and significantly higher on average for low NH_4 additions than controls, and significantly higher still for high NH_4 additions (Tukey's HSD, P < 0.05).

Temperature was virtually identical in enclosures with low and high NH_4 additions, while pH differed by less than 0.3 units on average. Temperature was highest on days 7 and 14, while pH varied from about 8.3 to 8.7 and showed no trend during the experiment in these enclosures (Figure A8). Values of pH showed no significant differences between treatments (RM-ANOVA, Wilk's $F_{8,24} = 0.96$, $P = 0.49$). Calculated percentages of unionized NH_4 were about 5–15% for all days, with no consistent differences among treatments. Salinity in all enclosures rose from initial values of approximately 0.71 g/L^{-1} to values of approximately 0.72 g/L^{-1} on day 14, and then dropped to values of approximately 0.69 g/L^{-1}. There were no significant differences between treatments (RM-ANOVA, Wilk's $F_{8,24} = 0.87$, $P = 0.55$). Thus, NH_4 additions did not detectably increase salinity in enclosures.

Nitrate data indicate that a small amount of nitrification may have occurred in enclosures with high NH_4 additions. Nitrate concentrations in controls and enclosures with low NH_4 additions remained below 0.3 μM on average throughout the experiment (Figure A5). In enclosures with high NH_4 additions, average nitrate concentration increased over the course of the experiment from 0.2 to 1.6 μM. Nitrate concentrations were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 14.16$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 0, no pairwise differences between means were significant (Tukey's HSD, $P > 0.05$). On days 7 and 14, nitrate was significantly higher on average for high NH_4 additions than in controls or enclosures with low NH_4 additions, and on day 21 nitrate was significantly higher on average in high NH_4 additions than in low NH_4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$).

The abundance of *P. parvum* increased throughout the experiment in controls and enclosures with high NH_4 additions (Figure 14). Control enclosures exceeded bloom levels of 10×10^6 cells/$liter^{-1}$ on day 14, while enclosures with low NH4 additions exceeded bloom levels on day 7. In contrast, the abundance of *P. parvum* increased from day 0 to 7 in enclosures with high NH_4 additions, and then declined until day 21, never exceeding bloom levels. Abundances of *P. parvum* were significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 17.45$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On days 0 and 7, the abundance of *P. parvum* was significantly lower on average for high NH_4 additions than for controls, and on days 14 and 21 the abundance of *P. parvum* was significantly lower on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). Although the abundance of *P. parvum* was

somewhat higher on day 21 with low NH_4 additions than in controls, consistent with a weak fertilizing effect, this difference was not statistically significant.

Acute lethal toxicity to fish displayed differing changes over time for different treatments (Figure 15). On day 7, enclosures with low and high NH_4 additions (LC50 about 20-25%) were more toxic to fish than controls (LC50 about 50%). On day 14, controls and enclosures with high NH_4 additions had comparable toxicity to fish (LC50 about 20-30%), and enclosures with low NH_4 additions were more toxic (LC50 about 6%). On day 21, enclosures with high NH_4 additions were considerably less toxic to fish (LC50 about 80%) than other treatments, controls had intermediate toxicity (LC50 about 30%), and enclosures with low additions of NH_4 were very toxic (LC50 about 3%). LC50 was significantly affected by treatments (RM-ANOVA, Wilk's $F_{6,24} = 18.05$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 14, however, no pairwise differences between treatments were significant (Tukey's HSD, $P > 0.05$). On day 7, LC 50 was significantly higher on average in controls than in high or low NH_4 additions, and on day 21, LC50 was significantly higher on average in high NH_4 additions than in low NH_4 additions or controls (Tukey's HSD, $P < 0.05$). Chronic sublethal toxicity to *Daphnia* was high in enclosures with low NH_4 additions, somewhat lower in controls, and lowest in enclosures with high NH_4 additions. *Daphnia* reproduction tested with samples from day 21 averaged 16 neonates (SD = 15) for controls, was zero for all enclosures with low NH_4 additions, and averaged 50 neonates (SD = 7) for enclosures with high NH_4 additions, with significant differences among treatments (ANOVA, $F_{2,6} = 2.23$, $P = 0.19$). *Daphnia* reproduction in enclosures with high NH_4 additions was significantly higher than in controls or enclosures with low NH_4 additions.

Chlorophyll *a* decreased from days 0 to 7 in all enclosures, and changed little thereafter in controls and enclosures with high NH_4 additions, but increased from days 14 to 21 in enclosures with low NH_4 additions (Figure 16). Throughout the experiment, chlorophyll *a* was higher on average in enclosures with NH_4 additions than in controls, consistent with a fertilization effect for total phytoplankton biomass. Chlorophyll *a* was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 59.78$, $P < 0.001$) on all days (ANOVA, $P < 0.05$). On day 0, chlorophyll *a* was significantly higher on average in low NH_4 additions than in controls, on days 7 and 14 chlorophyll *a* was significantly higher on average in low and

high NH_4 additions than in controls, and on day 21 chlorophyll *a* was significantly higher on average in low NH_4 additions than in high NH_4 additions, which were significantly higher than controls (Tukey's HSD, $P < 0.05$).

The total abundance of zooplankton decreased throughout the experiment in all enclosures, somewhat more rapidly in those with NH_4 additions (Figure 17). Total abundance of zooplankton was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 4.80$, $P = 0.001$) on day 7 (ANOVA, $P < 0.05$). On day 7, total abundance of zooplankton was significantly higher on average in low and high NH_4 additions than in controls (Tukey's HSD, $P < 0.05$).The total biovolume of zooplankton also decreased throughout the experiment in all enclosures, at similar rates among treatments. Total biovolume of zooplankton was significantly affected by treatments (RM-ANOVA, Wilk's $F_{8,24} = 6.07$, $P < 0.001$) on days 14 and 21 (ANOVA, $P < 0.05$). On day 14, total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in low NH_4 additions, and on day 21 total biovolume of zooplankton was significantly higher on average in high NH_4 additions than in controls and low NH_4 additions (Tukey's HSD, $P < 0.05$).

3 Conclusions and Recommendations

Conclusions

In the first experiment, conducted during a pre-bloom condition, all treatments proved effective against *P. parvum* accumulation and onset of toxicity (with the exception of the low NH_4 dosage). All three flushing levels prevented accumulation of cells to bloom proportions, as did both levels of lowered pH and the high NH_4 addition. Furthermore, these treatments appeared generally benign to other plankton. For example, flushing and lowered pH had no effect on total phytoplankton biomass. Addition of NH_4 stimulated phytoplankton biomass, however. Increased flushing caused lower zooplankton biomass at the highest flushing level only, while lowering of pH had no effect on zooplankton abundance. Addition of NH_4 led to reduced zooplankton biomass, although the variation in these data made it difficult to discern.

Similarly, all treatments proved effective against *P. parvum* accumulation and onset of toxicity in the second experiment, conducted during a period of bloom development and decline. All three flushing levels slowed the accumulation of *P. parvum* cells, with the highest level of flushing preventing bloom levels. Similarly, lowered pH slowed the accumulation of *P. parvum* cells, with pH levels of 7.5 and 7.0 preventing the bloom. Addition of NH_4 showed mixed results. The high dosage was lethal to *P. parvum*, while the lower dosage showed no effect on the accumulation of cells. Flushing resulted in decreased toxicity, as did the high NH_4 dosage. Lowering of pH to 7.0 completely eliminated toxicity. Addition of low NH_4 resulted in greater toxicity. Paralleling the first experiment, the treatments appeared generally benign to other plankton. Flushing and lowered pH again had no effect on total phytoplankton biomass, and addition of NH_4 stimulated phytoplankton biomass. With the exception of the low NH_4 addition, where toxicity was enhanced, treatments generally had no effect on zooplankton biomass.

Previous research founded in theory and system-wide monitoring showed that the incidence of *P. parvum* blooms was sensitive to the magnitude and timing of inflow events (Roelke et al. 2010a, 2011; Grover et al. 2011). *P. parvum* is sensitive to cell losses through hydraulic flushing because its reproductive growth rate is low when it forms blooms (winter).

Furthermore, toxin production is linked to nutrient availability (Roelke et al. 2007, Errera et al. 2008), where increased nutrient availability slows the toxin production rate. So, nutrient additions combined with hydraulic flushing should reduce toxin production. In the absence of toxin production, ambient toxicity decreases as a result of rapid photodecomposition of the toxins (James et al. 2011). Without the benefit of its toxins, *P. parvum* quickly loses its competitive advantage over other phytoplankton and is displaced. These results, based on in-lake experiments using natural assemblages at the mesocosm scale, further underscore the importance of hydraulic flushing as an influence on *P. parvum* blooms.

The toxicity of *P. parvum* is dependent on pH, as demonstrated in the *P. parvum* laboratory experiments using a clone established from Texas waters (Valenti et al. 2010). The toxins released by *P. parvum* seem to behave as weak bases in aqueous solutions that are more toxic at higher pH. The laboratory findings were supported by in-lake mesocosm experiments, where not only was toxicity ameliorated but *P. parvum* population density was prevented from reaching bloom proportions. Again, without the benefit of its toxins, *P. parvum* quickly loses its competitive advantage over other phytoplankton and is displaced. Because changes in pH can act at the chemical level (rather than indirectly through biologic effects), the impact of this manipulation may be more rapid, with observable effects after only 7 days.

P. parvum is sensitive to NH_4, specifically to the more toxic unionized form (Barkoh et al. 2003, Grover et al. 2007). This sensitivity is common in many phytoplankton taxa, where ammonia at high levels can inhibit both reproductive growth rates and rates of photosynthesis. In-lake mesocosm experiments were consistent with these previous findings, where *P. parvum* population density and toxicity were lessened at high NH_4 dosages. Inorganic nitrogen, however, is another nutrient essential for phytoplankton. Many phytoplankton taxa are able to utilize NH_4 directly as a nutrient, and most taxa are able to use nitrate, a product of nitrification that decreases NH_4 concentrations. So the lower level of NH_4 addition was not great enough to bring about an acute toxicity effect on *P. parvum;* instead, it increased nitrogen availability, allowing for greater accumulation of biomass.

These results suggest additional lines of inquiry. For example, what might result if such treatments were applied to a more open system, e.g., a cove

of a lake, instead of limnocorrals? If initiated during a pre-bloom period, would treatments have prevented a bloom? If initiated during a period of bloom development, would it be suppressed?

Recommendations

The incidence of *P. parvum* blooms has been linked to hydrology, and system-wide fish-killing blooms are observed only at times of low inflow (Roelke et al. 2010a, 2011). Unfortunately, stream flows are predicted to decrease in the southcentral United States as the climate changes and human population increases. Without mitigation, it is likely that the damage from these blooms will increase. To design and implement effective management strategies, further investigation, linked with in-field demonstration projects, is needed.

Manipulation of whole lake systems may not be practical. A focus on smaller areas, such as coves, seems more promising. Preventing blooms from forming or propagating into coves might create a refuge habitat for aquatic organisms, including the many recreationally important fish species in this region.

In-lake demonstrations that focus on flushing and pH neutralization are recommended. While NH_4 addition showed promise, downstream effects of fertilization have not been assessed. Flushing shows promise because water within the system free of *P. parvum* cells (deeper waters) can be used as the source water, so that waters from upstream locations would not be needed. pH neutralization shows promise because it works quickly and can be implemented at a minimal cost. Both flushing and pH neutralization were benign to other aquatic organisms and would likely have no downstream effects.

Lake Granbury would be an ideal location for such an in-lake demonstration. The lake has many coves from which appropriate locations could be selected and the ecology of the lake is relatively well understood from many years of study. In addition, *P. parvum* blooms are fairly predictable, so in-lake demonstrations could be timed well. Also significant here is strong local support from members of the Granbury community, who continue to engage with the scientific effort through town hall meetings and other avenues of communication.

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Appendix A: Approaches to Golden Algae Control: In-lake Mesocosm Experiments

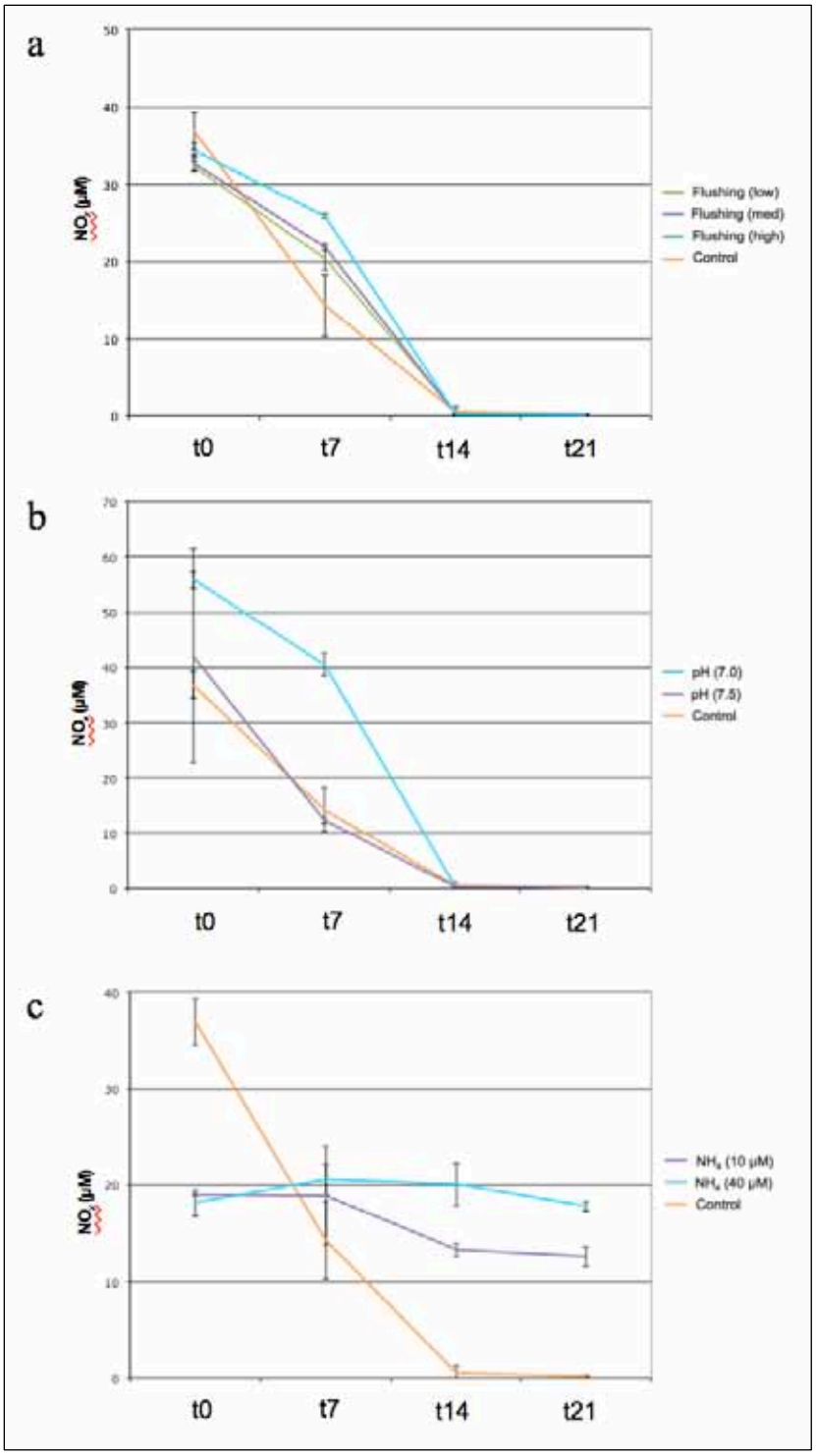

Figure A1. Time series data of NOX from weekly samplings of the pre-bloom experiment from enclosures with the flushing (a), pH (b), and NH_4 (c) treatments.

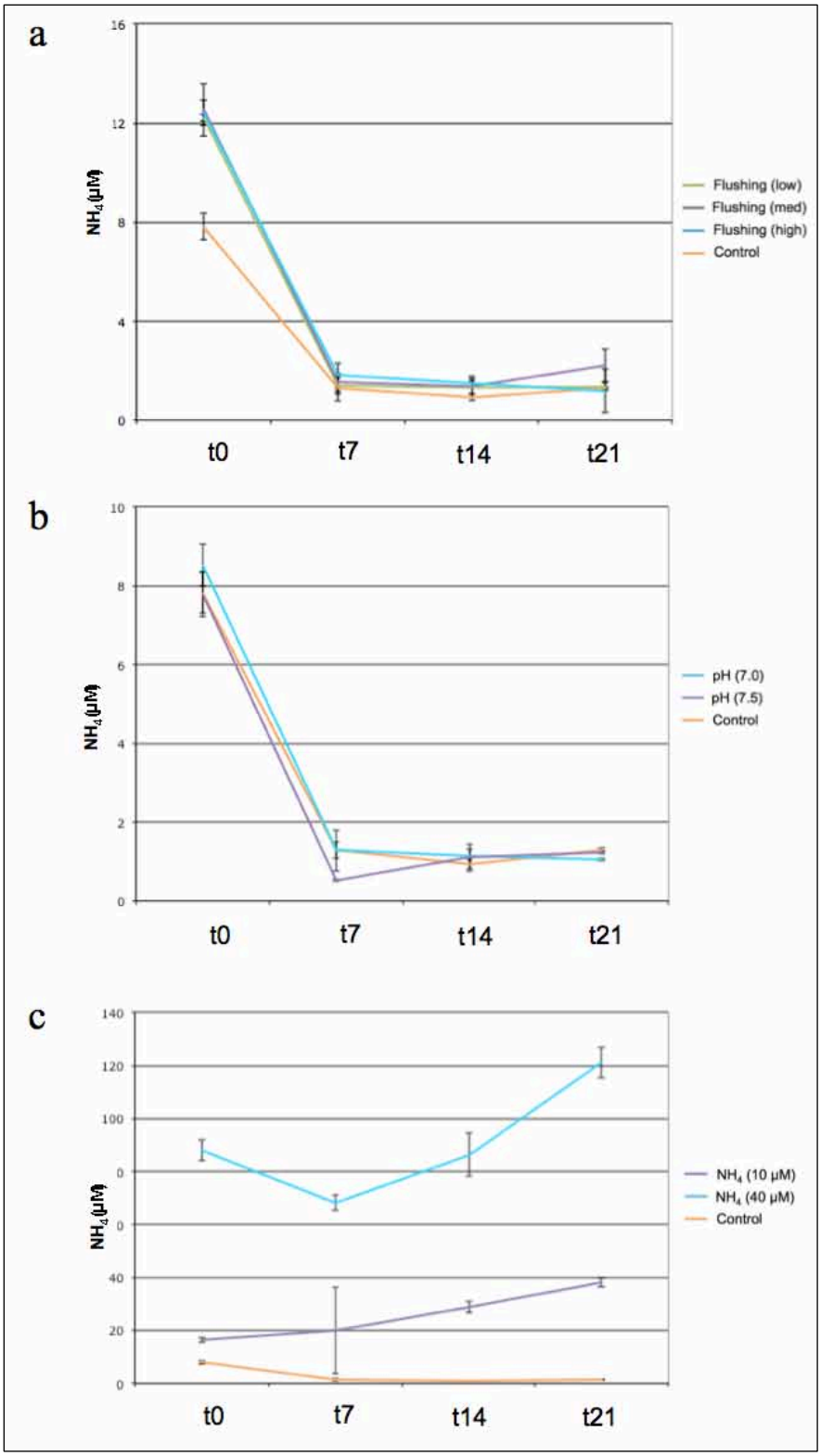

Figure A2. Time series data of NH_4 from weekly samplings of the pre-bloom experiment from enclosures with the flushing (a), pH (b), and NH_4 (c) treatments.

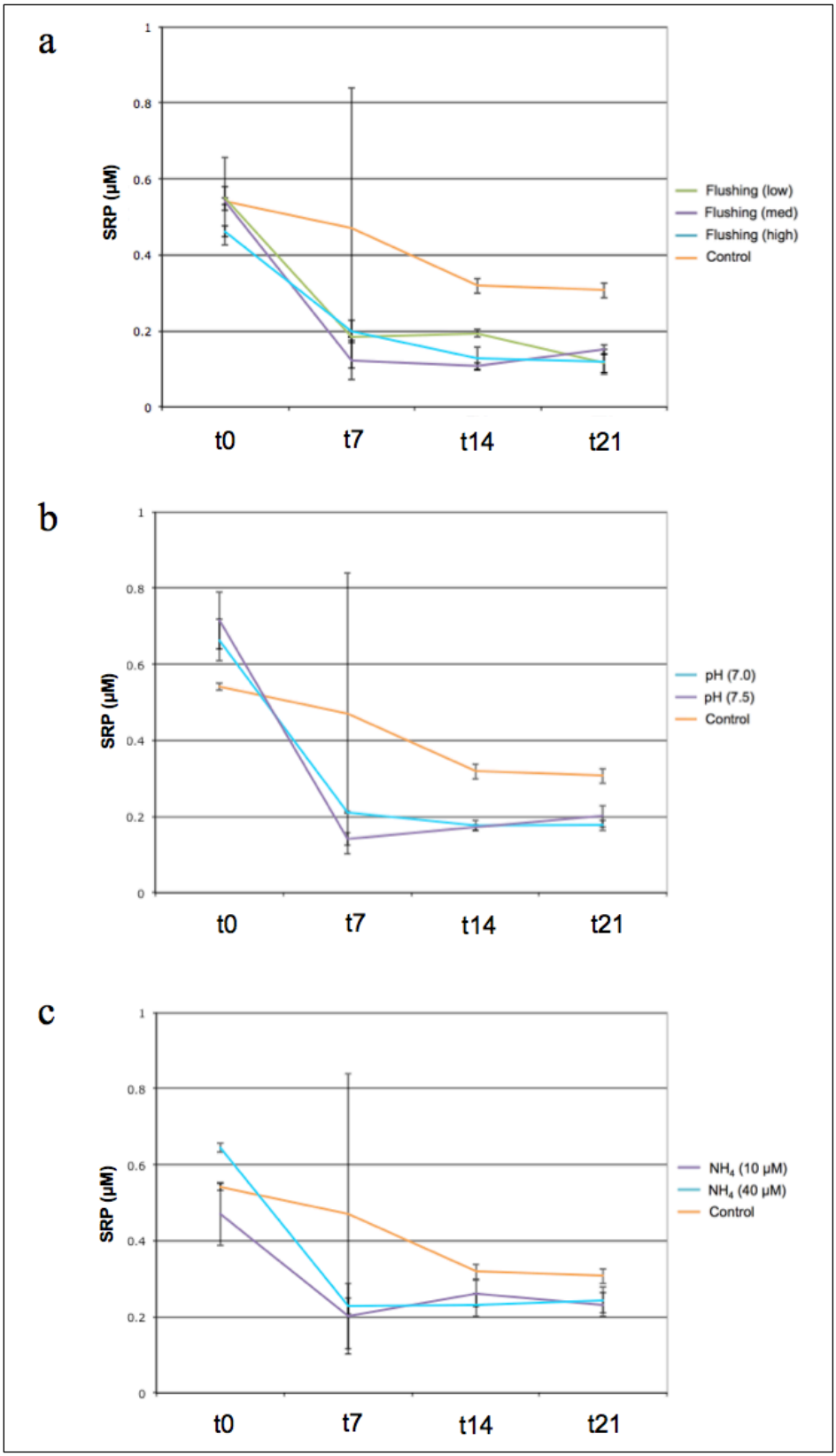

Figure A3. Time series data of PO4 from weekly samplings of the pre-bloom experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

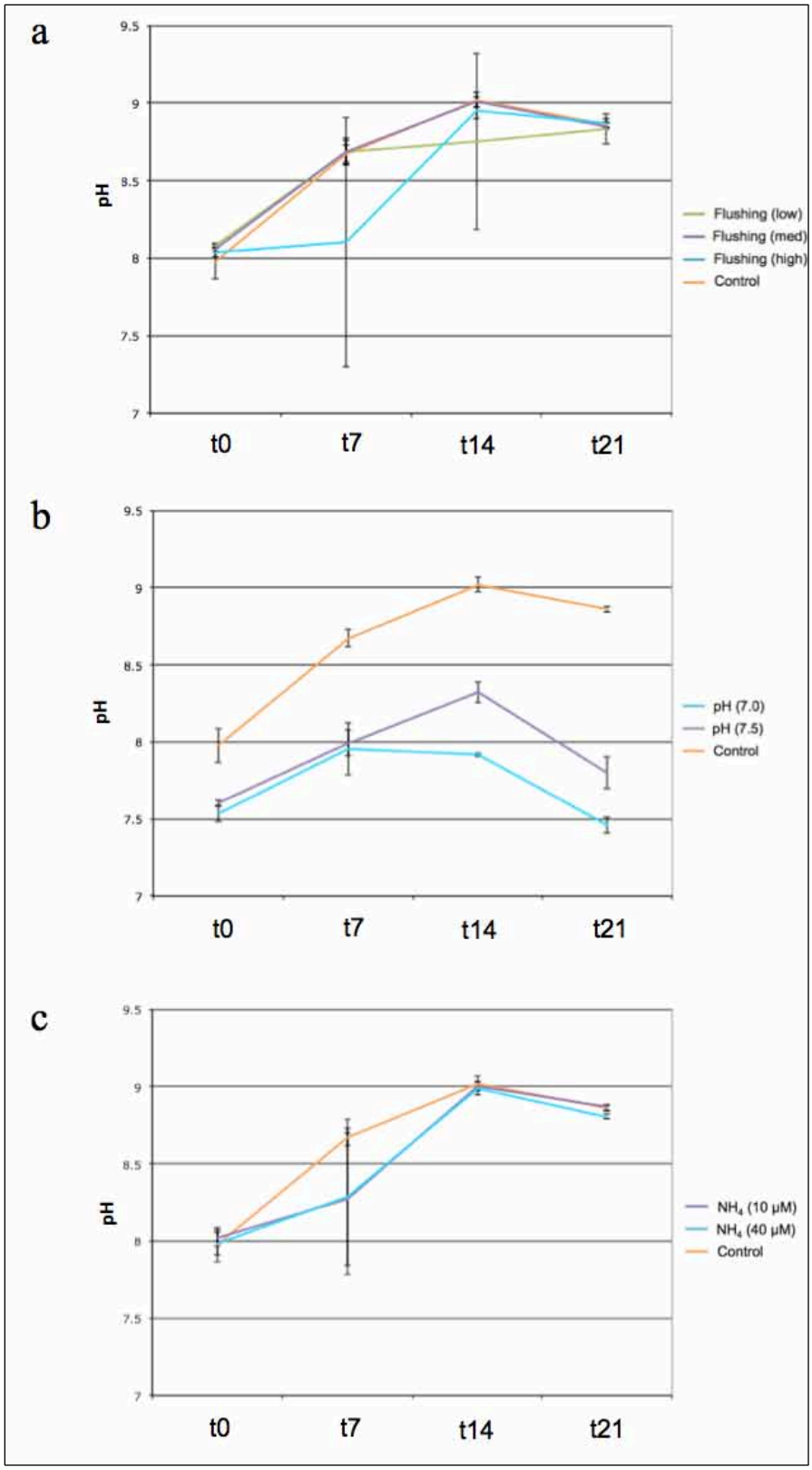

Figure A4. Time series data of pH from weekly samplings of the pre-bloom experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

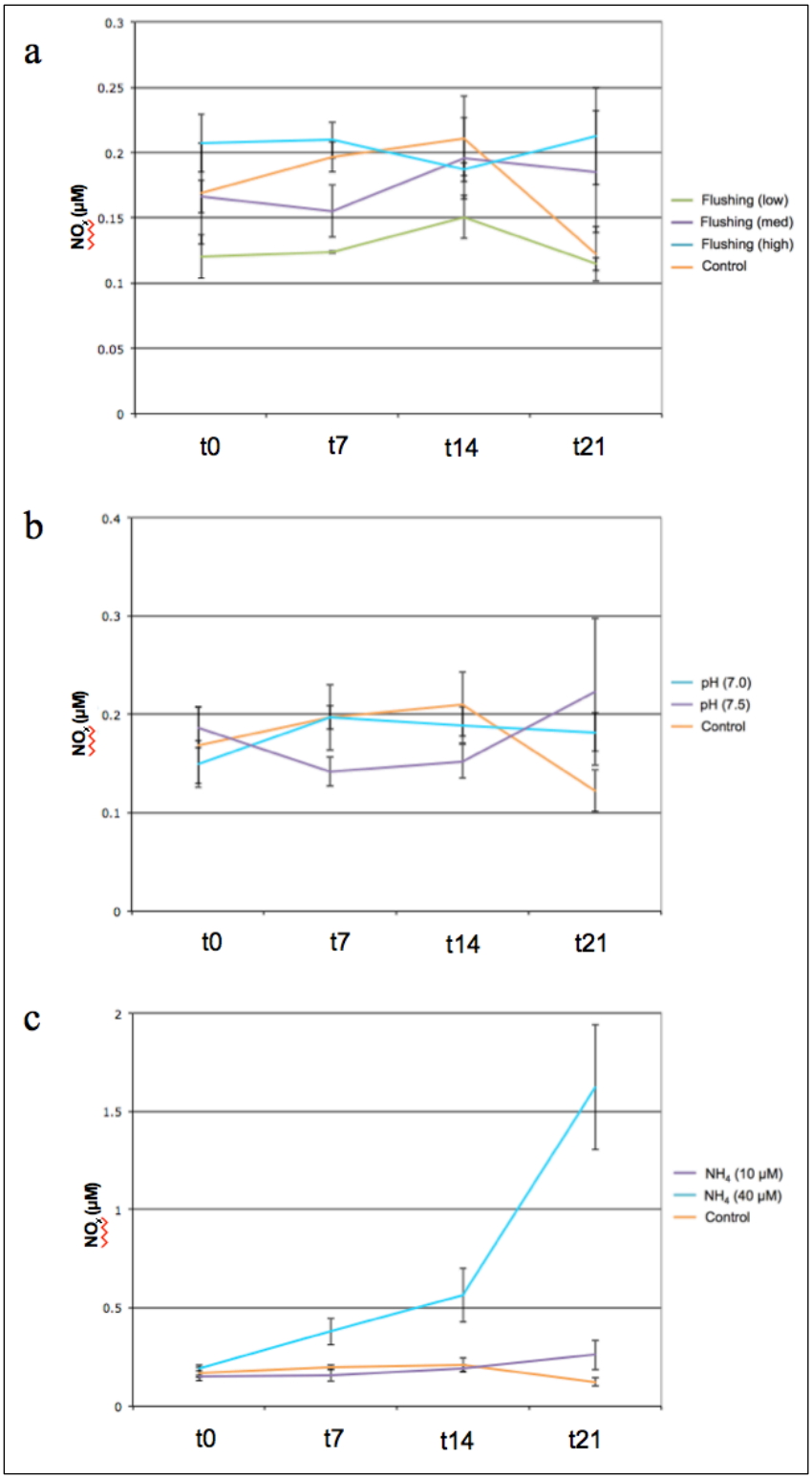

Figure A5. Time series data of NOX from weekly samplings of the bloom development experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

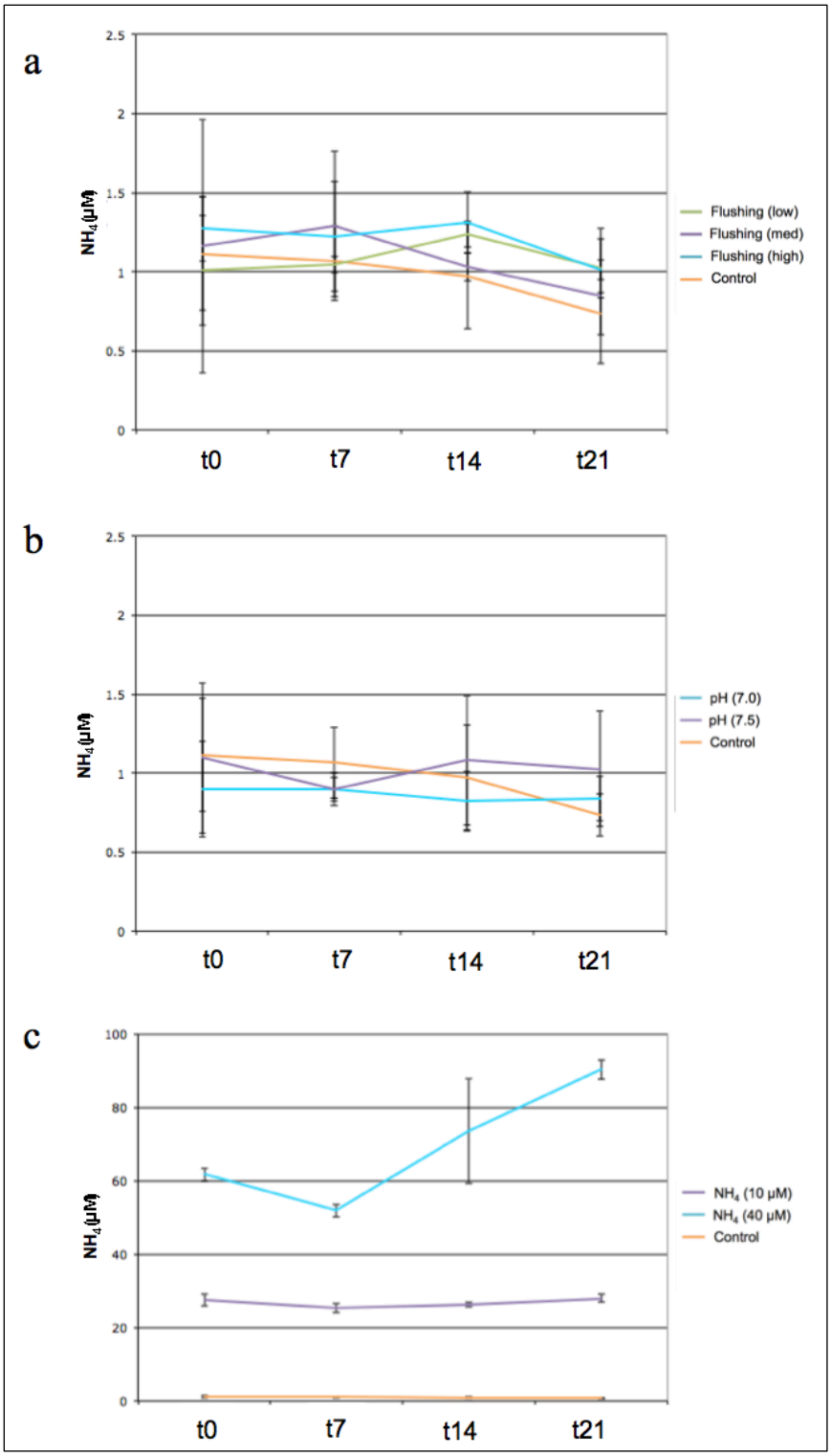

Figure A6. Time series data of NH4 from weekly samplings of the bloom development experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

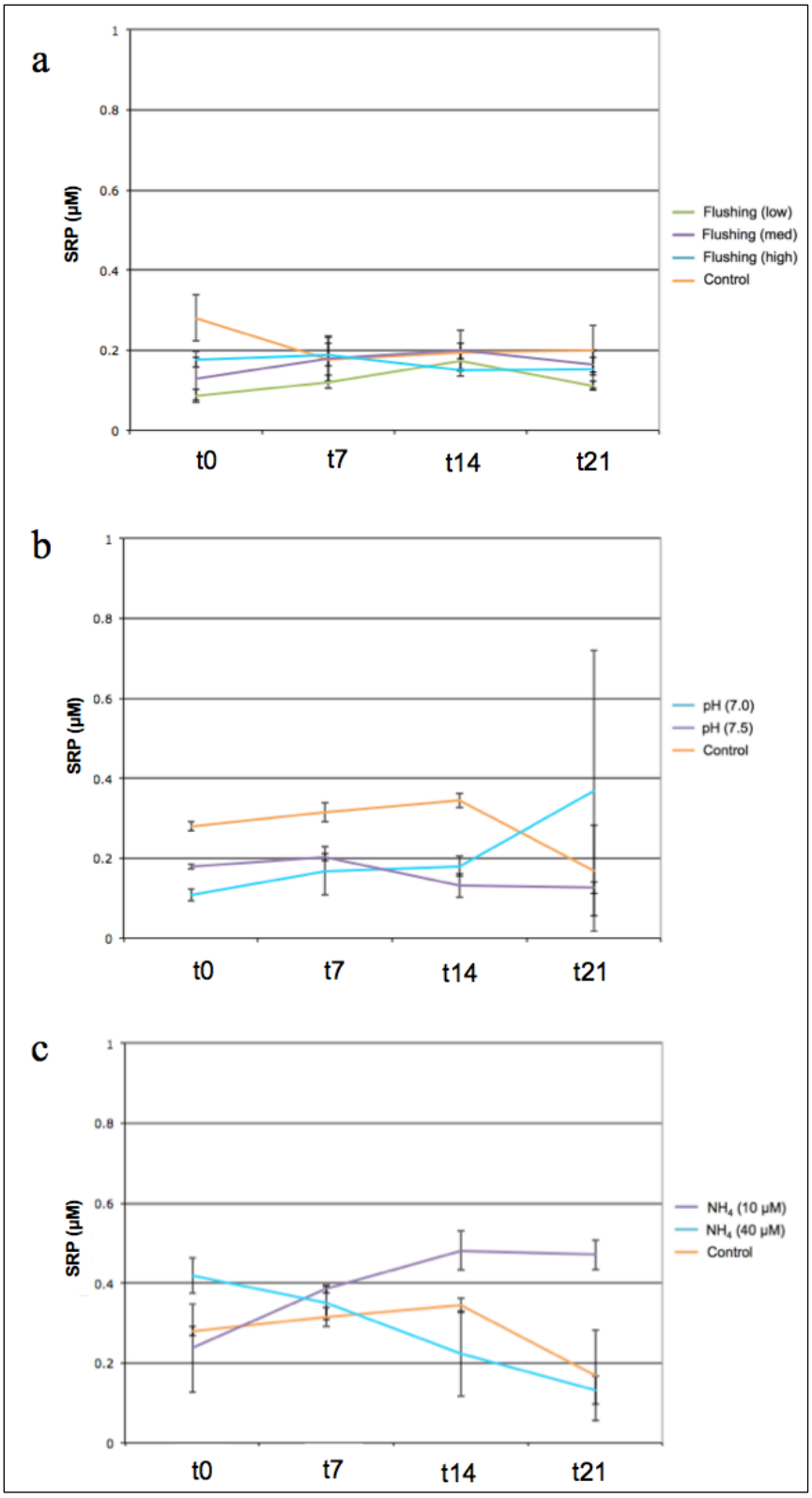

Figure A7. Time series data of PO4 from weekly samplings of the bloom development experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

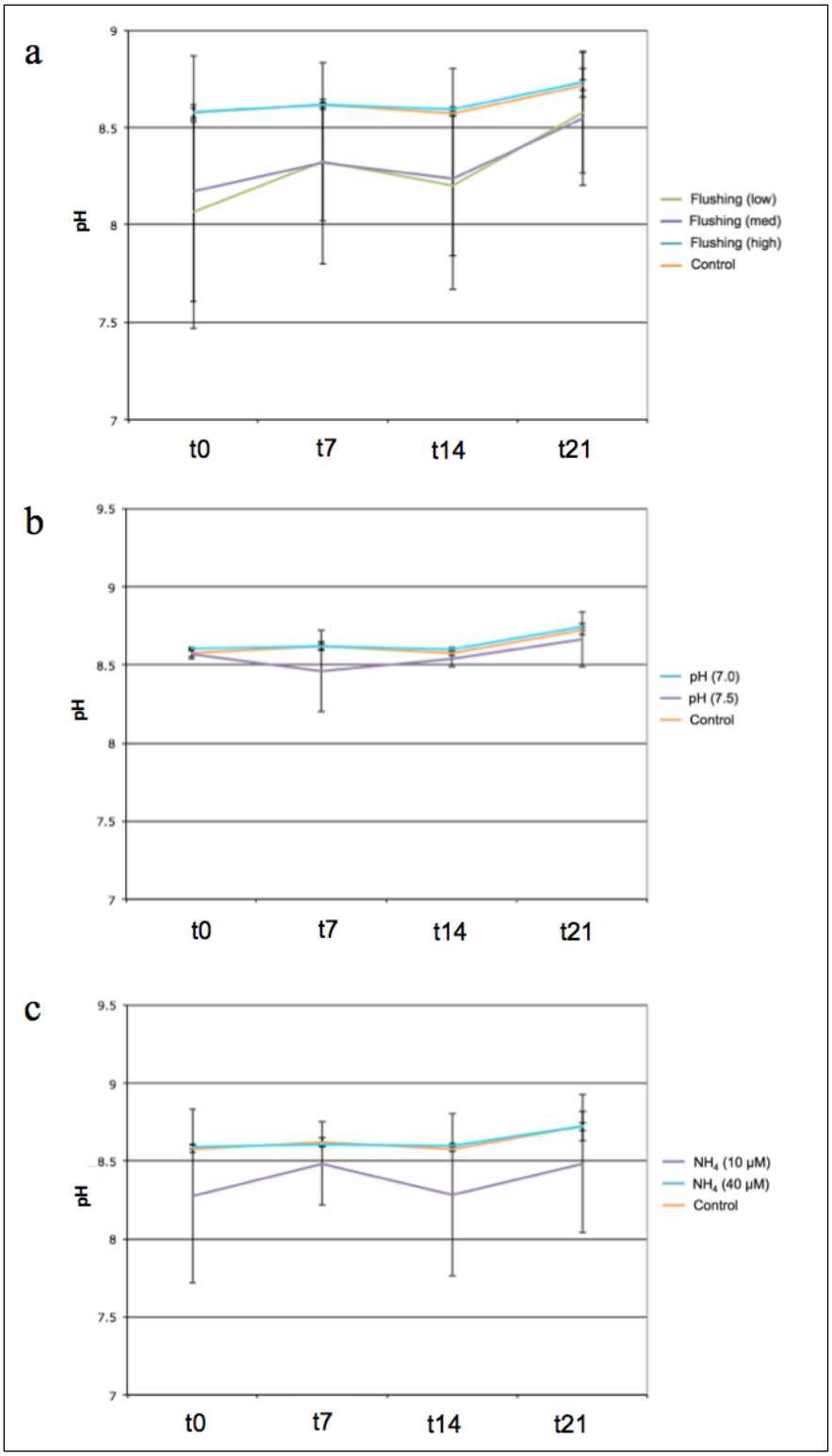

Figure A8. Time series data of pH from weekly samplings of the bloom development experiment from enclosures with the flushing (a), pH (b), and NH4 (c) treatments.

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14. ABSTRACT

Prymnesium parvum, a haptophyte alga, occurs worldwide. It is tolerant of large variations in temperature and salinity, and is capable of forming large fish-killing blooms. In the United States, the first recorded *P. parvum* bloom occurred in 1985 in a semi-arid region of the country (Pecos River, Texas). Since then, the reported incidence of *P. parvum* blooms dramatically increased in the United States, where the organism has invaded lakes and rivers throughout southern regions and most recently has moved into northern regions. Fortunately, *P. parvum* population dynamics are influenced by several factors that may serve as tools for management. These include hydraulic flushing, pH, and ammonia additions. This report documents in-lake enclosure experiments conducted during periods of bloom initiation, and bloom development and decline. Three approaches are demonstrated to have promise in controlling blooms in localized areas of lakes. Most promising were manipulations involving pulsed hydraulic flushing (30% water exchange once per week using water deeper in the lake); the neutralization of ambient waters (lowering pH to 7); and ammonia additions (elevating to 40 μM).

15. SUBJECT TERMS

Ammonia addition
Golden algae
Hydraulic flushing
pH addition
Prymnesium parvum

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